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(54) Title: DNA ENCODING OVINE ADENOVIRUS (OAV287) AND ITS USE AS A VIRAL VECTOR

## (57) Abstract

The invention relates to an isolated DNA molecule comprising the genome of ovine adenovirus OAV287, functionally equivalent DNA molecules or portions thereof. The invention also relates to plasmids and viral vectors including the DNA molecules. The invention also relates to methods for delivering non-adenoviral DNA molecules encoding a polypeptide or polypeptides to animals and in particular to grazing animals.

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DNA encoding ovine adenovirus (OAV 287) and its use as a viral vector

Technical Field

The present invention relates to a new full length genomic clone derived from a benign adenovirus (OAV287) isolated from sheep in Australia. The present invention also relates to new viral vectors derived from the benign ovine adenovirus and also relates to the use of these vectors for the delivery and expression of nucleic acid sequences encoding functional RNA molecules or polypeptides to animals.

Background of the Invention

Diseases caused by infectious agents and parasite infestations cause health problems and production losses in domestic animals but for many infectious agents no vaccine exists. Consequently, there are major research efforts worldwide to develop new vaccines which can protect against disease.

While some protective antigens from infectious agents and parasites have been identified, their successful use as vaccines requires the development of systems which can effectively deliver the antigen to the host. A variety of recombinant gene expression vectors derived principally from the pox virus family have been employed as these are generally of low pathogenicity. Expression of the foreign protein following infection by the recombinant viral vector may stimulate a protective immune response in the host.

However, no viral vector has all the attributes desirable for all situations. Some vectors are better suited to particular tasks than others because of their biological properties. For example, it has often proved difficult to stimulate an effective mucosal immune response which can protect against disease. In humans, adenoviruses have been given orally to vaccinate against respiratory disease (1). As this involves protection at mucosal surfaces adenoviruses clearly have potential in

this regard. Human adenovirus vectors have also been used to deliver genes to muscle (2) and other tissues.

Although adenoviruses do not generally integrate their DNA into the cellular genome, nevertheless, the DNA persists and long term protein expression is observed. Expression of an appropriate antigen from such cells can generate a systemic immune response which may be protective against the homologous disease causing agent.

Known adenovirus genomes are linear double-stranded DNA molecules which have an inverted terminal repeat sequence (ITR) at each end and a protein covalently bound to the 5'-terminal C residue (3). The genome sequence and structure has now been completely determined for human adenoviruses types 2, 5, 12 and 40 and partially for numerous others, including some animal isolates (see Genbank and EMBL Nucleic Acid databases). Human adenovirus type 2 was the first genome to be sequenced but broadly speaking its genome arrangement is conserved among other characterized adenoviruses i.e. early regions E1-E4 and the structural protein homologues can be recognized in similar locations in the genome. In particular, the E1A/E1B region is located at the left hand end of the genome and region E4 is always located at the right hand end of the genome. Early region E3 is always located between the genes for structural proteins pVIII and fiber, although its size and complexity varies between species e.g. from 3kb with at least 10 open reading frames in human adenoviruses to approximately 0.7kb with only two significant open reading frames in murine adenovirus (4, 5). E3 is a key region for the construction of recombinant viruses as it is non-essential for replication in vitro (6). The late, L region is expressed from the major late promoter, MLP and complex splicing generates families of mRNAs which code for most of the structural viral proteins. Proteins IVa2 and IX appear to have their own promoters.

Although there are some human viral vectors available for medical use there are few animal viral vectors suitable for use in veterinary applications. In order to obtain a more suitable animal viral vector the present inventors have purified an ovine adenovirus (OAV287) isolated from sheep in Western Australia. This ovine adenovirus is serologically related to bovine adenovirus type 7 but is genetically distinct from the bovine adenoviruses and other Australian ovine isolates, as shown by comparisons between the ovine and bovine adenoviruses, based on restriction enzyme profiles (8). The genome arrangement of the virus according to the present invention varies significantly from all other known adenoviruses. The adenoviral DNA molecule of the present invention is suitable for use in viral vectors capable of expressing a variety of polypeptides when used for veterinary applications.

#### Summary of the Invention

According to a first aspect, the present invention consists in an isolated DNA molecule comprising a nucleic acid sequence encoding the genome of ovine adenovirus (OAV287) substantially as shown in Figure 1 or a functionally equivalent nucleic acid sequence. Preferably, the nucleic acid sequence encoding the genome of the adenovirus is substantially as shown in Figure 1.

In a further preferred embodiment of the first aspect of the present invention, the DNA molecule comprises a nucleic acid sequence encoding the genome of ovine adenovirus (OAV287) wherein a portion of the adenoviral genome not essential for the maintenance or viability of the native adenovirus deleted or altered.

In a second aspect, the present invention consists in a DNA molecule including at least a fifteen nucleic acid base sequence being substantially unique to the ovine adenovirus (OAV287) nucleic acid sequence shown in Figure 1. In a preferred embodiment of the second aspect of the

present invention, the at least fifteen nucleic acid base sequence encodes a functional element of ovine adenovirus (OAV287). Preferably, the functional element is selected from the group consisting of promoter, gene, inverted terminal repeat, viral packaging signal and RNA processing signal. The inverted terminal repeat of ovine adenovirus (OAV287) comprises the first 46 nucleic acid bases from the 5' end of each strand of the double stranded DNA genome of the virus.

In a third aspect, the present invention consists in a plasmid including the DNA molecule of the first or second aspects of the present invention. Preferably, the plasmid includes the DNA molecule of the first aspect of the present invention wherein the nucleic acid sequence encoding the adenoviral genome is linked to a nucleic acid sequence encoding an origin of replication and a further nucleic acid encoding a marker. Preferably, the nucleic acid sequence encoding the marker encodes for resistance to an antimicrobial agent. More preferably the antimicrobial agent is ampicillin.

In a further preferred embodiment of the third aspect of the present invention, sequences encoding inverted terminal repeats of the adenovirus are joined.

In a fourth aspect, the present invention consists in a viral vector comprising the DNA molecule of the first aspect of the present invention and at least one nucleic acid sequence encoding a non-adenoviral polypeptide or polypeptides.

Preferably, nucleic acid sequence encoding the non-adenoviral polypeptide or polypeptides is derived from bacteria, viruses, parasites or eukaryotes. More preferably, the non-adenoviral polypeptide is rotavirus VP7sc antigen, the parasite polypeptide is *Trichostrongylus colubriformis* 17kD antigen, the *Taenia ovis* 45W antigen or the PM95 antigen from *Lucilia cuprina*.

In another form, the present invention consists in a viral vector comprising the DNA molecule of the first aspect of the present invention and at least one nucleic acid sequence encoding a functional RNA molecule. It will be appreciated by one skilled in the art that a functional RNA molecule can include a messenger RNA molecule, an antisense RNA molecule or a ribozyme.

In a fifth aspect, the present invention consists in a method of delivering a DNA molecule having a nucleic acid sequence encoding a non-adenoviral polypeptide or polypeptides to a target cell comprising infecting the target cell with a viral vector according to the fourth aspect of the present invention such that the DNA molecule encoding the polypeptide or polypeptides is expressed and the polypeptide or polypeptides is produced by the target cell.

In a sixth aspect, the present invention consists in a method for delivering a DNA molecule having a nucleic acid sequence encoding a non-adenoviral polypeptide or polypeptides to an animal comprising administering to the animal a viral vector according to the fourth aspect of the present invention such that the viral vector infects at least one cell of the animal and the infected cell expresses the DNA molecule encoding the polypeptide or polypeptides and produces the polypeptide or polypeptides. Preferably the animal is a grazing animal and more preferably the grazing animal is a sheep.

In another form, the present invention consists in a method for delivering a DNA molecule having a nucleic acid sequence encoding a functional RNA molecule to an animal comprising administering to the animal a viral vector of the fourth aspect of the present invention having a nucleic acid sequence encoding a functional RNA molecule such that the viral vector infects at least one cell of the animal and the infected cell expresses the DNA

molecule encoding the functional RNA molecule and produces the functional RNA molecule.

As used herein the term "functionally equivalent nucleic acid sequence" is intended to cover minor variations in the ovine adenovirus (OAV287) DNA molecule which, due to degeneracy in the DNA code, does not result in the molecule encoding different viral polypeptides. Further, this term is intended to cover alterations in the DNA code which lead to changes in the encoded polypeptides, but in which such changes do not substantially affect the biological activities of these viral polypeptides.

As used herein the term "functional element" is intended to cover nucleic acid sequences that encode promoters, genes, inverted terminal repeats, viral packaging signals and RNA processing signals. It will be appreciated by one skilled in the art that unique sequences from ovine adenovirus (OAV287) that encode these functional elements may be useful in other systems including plasmids and non-ovine adenoviral vectors.

In order that the nature of the present invention may be more clearly understood preferred forms thereof will be described with reference to the following examples and the accompanying drawings.

#### Brief Description of the Drawings

Figure 1 is the nucleic acid sequence of the OAV287 genome beginning at base 1 of the left-hand ITR.

Figure 2 shows the arrangement of OAV287 genes based on homologies detected with Ad2. Regions with question marks are tentative identifications because of the lack of obvious homology.

Figure 3 indicates the major open reading frames in the proposed E1 region of OAV287. Asterisks show the location of possible initiation codons. A previously unidentified gene (p28kD) which codes for a processed structural protein is encoded on the complementary strand.



Figure 4 shows open reading frames in the region of the OAV287 expected to contain E3. However, E3 is missing as the gap between the pVIII and fiber genes is only 197 nucleotides. The site at which the ApaI/NotI polylinker was later inserted is indicated.

Figure 5 shows the major open reading frames in the probable E3 region of OAV287. Asterisks show the location of potential initiation codons. The SalI site which was modified by end-filling and re-ligation and the alternative site at which a polylinker sequence was later inserted into the genome without loss of infectivity is indicated.

Figure 6 is a scheme describing the construction of a plasmid (pOAV287Cm) containing a full-length clone of the OAV287 genome with pACYC184 sequences inserted in the SalI site. Filled in regions show OAV287 sequences. Cross-hatched sequences are derived from plasmids pUC13 or Bluescribe M13+ (Amp<sup>R</sup>), stippled regions from pSELECT (Tet<sup>R</sup>) and open regions from pACYC184(Cm<sup>R</sup>). Only the key restriction sites used for plasmid construction are indicated.

Figure 7 shows a map of the plasmids pOAV100, pOAV200, pOAV600 and pOAV600S. Arrowheads indicate the ITRs and the approximate location of the major late promoter (MLP). The mutated SalI site and sites at which the ApaI/NotI polylinker sequences were inserted are indicated. Light hatching signifies modified Bluescribe sequences inserted in the KpnI site. Linear, infectious genomes (dark hatching) are released by digestion with KpnI.

Figure 8 shows the results of screening ovine adenoviruses OAV100 and OAV200 rescued by transfection of recombinant plasmids pOAV100 and pOAV200 into CSL503 cells. Portions of the genome spanning (A) the mutated SphI site in OAV100 and (B) the ApaI/EcoRV/NotI polylinker insertion site in OAV200 were amplified by PCR together

with the corresponding regions from wild-type OAV287. The products were digested with SphI (A, lanes 3 & 5) and ApaI, EcoRV or NotI (B, lanes 3-5, and 8-10, respectively). (U) indicates undigested samples.

5        Figure 9 is a map of a plasmid pMT used for the assembly of gene expression cassettes. Fragments containing the OAV287 major late promoter and tripartite leader sequences are linked and precede a multiple cloning site for the insertion of genes of interest. A tandem  
10 polyadenylation signal (AATAAA) follows.

Figure 10 shows a summary of recombinant viruses which have been rescued from the corresponding infectious plasmids and the gene expression cassettes they carry. Cassettes were inserted into the OAV genome between the  
15 pVIII and fibre genes as indicated.

Figure 11 shows the expression of (A) the *T. ovis* 45W and *L. cuprina* PM95 antigens in CSL503 cells following infection of these cells with OAV205 and OAV210 viruses, respectively and (B) VP7sc expression in CSL503 and bovine  
20 nasal turbinate cells following infection with virus OAV204. (I) Infected cells (U) Uninfected cells. (M) indicates marker proteins of the sizes shown.

Figure 12 shows expression of VP7sc in (A) CSL503 cells and (B) rabbit kidney and bovine nasal turbinate  
25 cells following infection with OAV206 virus. (I) Infected cells. (U) uninfected cells. (M) indicates marker proteins of the sizes shown.

### Description of the Invention

#### METHODS

##### 30    Growth and Purification of OAV287

The virus, isolated from sheep in 1985, was obtained from R.L. Peet, Animal Health Laboratory, Department of Agriculture, Western Australia. The virus isolate was grown in sheep foetal lung cells (line CSL503) and twice  
35 plaque-purified under solid overlay before stocks were prepared. Virus was purified from CSL503 cells as

described previously (18, 22). DNA was extracted from the virus by digestion with proteinase K (23).

#### Cloning of Genome Fragments

Molecular techniques for manipulation, modification and transformation of plasmid DNA which were used in the work described below are described in (9) and similar publications. OAV287 DNA was digested with various restriction endonucleases including BamHI, SphI, SmaI and SalI to deduce the location of these sites (18).

The adenovirus genome has a protein covalently linked to each end of the linear dsDNA (24). The BamHI A and D fragments of approximately 8kb and 4kb, respectively, were identified as the terminal genomic fragments because their migration into agarose gels was dependent on the pre-digestion of viral DNA with proteinase K. The internal BamHI fragments B, C, E and F, estimated at 6.2, 5.1, 3.4 and 1.1kb in size respectively, were separated on an agarose gel, recovered and cloned into BamHI-digested pUC13 using standard ligation and transformation procedures (9). To clone the terminal BamHI A and D fragments, viral DNA (10µg) was digested with proteinase K (50µg/ml in 10mM Tris/HCL, pH8.0, containing 1mM EDTA and 0.5% SDS) at 65°C for 60min to remove the terminal protein. The DNA was extracted twice with phenol/chloroform, once with ether and recovered by ethanol precipitation. The 3'ends (of unknown sequence) were then digested exo-nucleolytically with T<sub>4</sub> DNA polymerase (5 units, Toyobo, Tokyo, Japan) in the presence of dATP (100µM) in buffer containing Tris HCL (50mM), pH8.0, MgCl<sub>2</sub> (7mM), 2-mercaptoethanol (7mM) and BSA (10µg/ml) for 15min at 37°C. The DNA was again purified by phenol extraction and ethanol precipitation described above. To remove the single-stranded terminal regions and create blunt ends the DNA was digested with 1 unit of mung bean nuclease (Pharmacia, North Ryde, Australia) for 10 min at 37°C in buffer containing Na acetate (30mM), pH4.6,

NaCl (50mM) and ZnCl<sub>2</sub> (1mM) before extraction with phenol/chloroform and recovery by ethanol precipitation. Finally the DNA was digested with BamHI (Pharmacia) and the fragments were separated by electrophoresis in low-melting-point agarose. The BamHI A and D fragments were excised, recovered by NACS column chromatography (BRL, Gaithersburg, Md) and ligated with BamHI/HincII-cut plasmid Bluescribe M13<sup>+</sup> (Stratagene, La Jolla, Ca) prior to transformation into *E. coli* JM109. Positive clones carrying fragments of the expected size were identified, restriction digested and confirmed as correct by nucleotide sequencing and comparison with partial sequence determined directly from genomic DNA. This revealed that three 3'-terminal nucleotides were removed during the cloning procedure.

#### Nucleotide Sequencing of the OAV287 Genome

The complete sequence of the OAV287 genome was determined by sequencing the BamHI fragments A-F using the Sanger method (25) and various kits provided by commercial suppliers. Nested deletions were constructed for the five largest fragments using a double-stranded nested deletion kit (Pharmacia). These were sequenced using standard primers. Based on newly determined sequence other nucleotide primers were synthesised using a DNA synthesizer (ABI, Model 391). In this way both strands of the entire genome and the junctions between the fragments were sequenced.

#### Mutagenesis of the OAV287 genome

For the construction of a full length OAV287 clone and subsequent modification of it to create plasmids such as pOAV200 and pOAV600 certain mutations were required. A relevant portion of the genome was subcloned into Bluescribe (Stratagene, La Jolla, Ca) or a similar plasmid which allowed rescue of single stranded DNA. Later it became possible to use dsDNA for mutagenesis. Oligonucleotides of the desired sequence were synthesized,

phosphorylated and used as primers as described by the manufacturers of Muta-gene Phagemid (Biorad Labs, Ca) or Altered sites II (Promega, Wi) mutagenesis kits. Mutations were generally identified by digestion with the appropriate restriction enzyme or by nucleotide sequencing, or both. Genome fragments containing introduced mutations were subcloned to create larger plasmids such as pOAV200 using appropriate unique restriction sites.

10 Construction of a Full-Length Genomic Clone of OAV287

The terminal BamHI A and D fragments (cloned in Bluescribe M13<sup>+</sup>) were each modified by mutagenesis to add the nucleotides lost during cloning and a KpnI site. The last base of the KpnI site incorporated the C at the 5' end of each genomic ITR sequence. This produced plasmids pAK and pDK (Figure 6).

The left hand approximately 21.5kb of the genome was constructed from the BamHI D and B fragments and the SphI A fragment of approximately 13kb. The genomic BamHI B fragment cloned in pUC13 was modified by mutagenesis (GCATGC to GCATCC) to remove the SphI site at position 8287 producing pUC13B. The modified fragment was released by BamHI digestion and cloned into pDK which had been cut with BamHI and dephosphorylated. Colonies carrying the recombinant plasmid pDBM (Figure 6) were identified by screening with an oligonucleotide which spanned the BamHI B/D junction. The SphI A fragment (approximately 13kb) was cloned into the SphI site of pSELECT (Promega) to form pSESPH. This fragment contains a SmaI site near its left hand end which is common to pDBM. The KpnI/SmaI fragment from pDBM was subcloned into pSESPH which had also been cut with KpnI/SmaI to produce pSELLH, a plasmid based on pSELECT which now contained the left-hand approximately 21.5kb of OAV287 DNA.

35 The right-hand end of the genome was constructed from pAK which contains the right-hand approximately 8.6kb

of the genome and overlaps the SphI A fragment. pAK was cut with SalI and ligated with SalI-cut pACYC184, a plasmid of 4.24kb which contains a gene encoding chloramphenicol (Cm) resistance and an origin for DNA replication, to form a pACm (Figure 6). This plasmid was cut with SphI and KpnI to produce the right-hand genomic fragment incorporating the pACYC184 sequences. This was ligated with the left-hand KpnI/SphI fragment of approximately 21.5kb prepared from pSELLH to produce the final plasmid pOAV287Cm (Figure 6). This plasmid replicates stably in E. coli and therefore removes the need to propagate the virus to obtain genomic DNA for further study. The recombinant genome in plasmid pOAV287Cm differs from the wild-type viral genome by the single point mutation in the SphI site (base 8287), by the presence of pACYC184 sequences in the SalI site and by the addition of a GTAC sequence between the ITRs. However, insertion of pACYC184 sequences in the SalI site disrupts two significant open reading frames whose functions are unknown. If either of the gene products was essential for replication, then pOAV287Cm could not produce infectious virus following transfection. To circumvent this potential problem pOAV287Cm was modified further. First, plasmid Bluescribe M13- (Stratagene, La Jolla, Ca.) was cut with HindIII and end-filled. The linear plasmid was then cut with SmaI, blunt-end ligated and transformed. The resulting plasmid contained an ampicillin resistance gene and origin of replication and lacked SalI and SphI sites but retained a unique KpnI site. This plasmid was cut with KpnI and ligated with KpnI-cut pOAV287Cm. Plasmids which were doubly resistant to ampicillin and chloramphenicol were selected and grown. One of these was cut with SalI to release the pACYC184 sequences, religated and transformed. The resulting plasmid pOAV100 contained the AmpR gene and replication Ori inserted in the KpnI site between the ITR's of the genome (Figure 7). This

plasmid replicated stably in *E. coli* strain JM109 when maintained in the presence ampicillin (200µg/ml). Large quantities of plasmid were grown for transfection studies. Transfection of DNA and Virus rescue

5           To determine whether the recombinant genomic clone was infectious, pOAV100 was cut with KpnI to release the linear viral genome and DNA was transfected into CSL503 sheep foetal lung cells using lipofectamine (GibcoBRL). Solution (A) containing plasmid DNA (2-10µg) and 300µl  
10       EMEM (containing hepes + glutamine), but lacking foetal calf serum (FCS) and solution (B) containing lipofectamine (10µl) + 300µl EMEM (containing hepes + glutamine), but lacking FCS were combined, mixed gently and incubated for 45 minutes at room temperature. Subconfluent CSL503 cells  
15       in a 60mm petri dish were rinsed with 3ml EMEM (plus hepes and glutamine) lacking FCS. EMEM (plus hepes and glutamine) but lacking FCS (2.4ml) was added to the mixture of solutions A and B, mixed gently and added to the rinsed CSL503 cells. Cells were incubated for 5 hours  
20       at 37°C in 5% CO<sub>2</sub>. The incubation medium was changed using complete EMEM plus FCS (10%) and cells were incubated at 37°C in 5% CO<sub>2</sub> until virus plaques or cytopathic effect was visible (7-15 days).

          To confirm that viruses rescued from transfection of  
25       pOAV100 and pOAV200 were derived from those plasmids a portion of the genome of wild-type OAV287, OAV100 and OAV200 viruses was amplified by PCR. For OAV100 a primer pair spanning the region of the mutated SphI site at bases 8287-8292 was used. For OAV200 the primer pair spanned  
30       the insertion site for the ApaI/NotI polylinker between the pVIII and fiber genes. Wild-type OAV287 DNA was amplified as a control in each case. DNA amplified from wild-type OAV287 was cut with SphI whereas the DNA amplified from OAV100 was not (Figure 8A). Similarly  
35       OAV200 DNA was cut with ApaI, EcoRV and NotI whereas

OAV287 DNA was not (Figure 8B). Other viruses were similarly characterised by restriction enzyme digestion. Identification of MLP/TLS elements and Construction of pMT

OAV287 TLS elements were identified as follows and  
5 as described (17). mRNAs present in OAV287-infected  
CSL503 cells were copied into cDNA by reverse  
transcription using primers complementary to the IIIa or  
fiber genes. A primer thought to fall within TLS exon 1  
was then paired with each cDNA primer for PCR. DNA was  
10 successfully amplified, cloned and sequenced. This  
identified TLS exons 2 and 3 (which correspond to bases  
8083-8145 and 8350-8412 of Figure 1, respectively) and the  
3' boundary of TLS exon 1 which occurs at base 5044 of  
Figure 1. A second PCR strategy was then used to obtain  
15 MLP and TLS fragments suitable for assembly into pMT. The  
region in Figure 1 between nucleotides 4861 and 5023,  
thought to contain the MLP was amplified by PCR using a  
plus sense primer which added an ApaI sequence at the 5'  
end and a 3' minus sense primer which introduced an NdeI  
20 site by point mutation at base 5012. Similarly, the TLS  
was amplified using a plus sense primer which introduced  
the NdeI site at base 5012 and a minus sense primer which  
was complementary to bases 8396-8412 and which added a  
HindIII site at the 3' end of the PCR product. The PCR  
25 fragments were digested with ApaI/NdeI and NdeI/HindIII,  
respectively and the fragments were cloned into Bluescript  
SK+ (Stratagene) cut with ApaI/HindIII. The resulting  
plasmid was then digested with HindIII/NotI and a  
synthetic oligonucleotide with HindIII/NotI termini and  
30 the sequence shown in Figure 9 was cloned to produce  
plasmid pMT. Genes of interest were then cloned into  
convenient restriction sites in the NCS. Gene expression  
cassettes were subcloned as ApaI/NotI fragments into  
pOAV200 or rescued into infectious virus.



### Infection of cells and expression of antigens

CSL503 and other cells were infected with viruses at a multiplicity of infection of 20pfu/cell as described previously (21). Infection was allowed to proceed for 24-60 hr. Cells were then incubated in methionine-free medium in the presence of  $^{35}\text{S}$ -methionine to label newly synthesized proteins. The protein of interest was recovered from cell lysates by immunoprecipitation using a specific antiserum against the expressed protein (21). Recovered proteins were analysed by polyacrylamide gel electrophoresis and detected by autoradiography or using a phosphorimager (Molecular Dynamics).

### RESULTS

To characterise the genome in molecular terms, BamHI restriction fragments representing the entire OAV287 genome were cloned into various plasmids and sequenced using methods described in Sambrook (9) and similar publications. Sequences were determined on both strands by using nested sets of deletion mutants together with synthetic oligonucleotide primers which were synthesized from newly determined sequences.

The viral sequence of 29,544 nucleotides (Figure 1) is considerably shorter (by approximately 6.5kb) than the sequence for human adenoviruses but many genes encoding structural proteins are identified by their homology with their Ad2 homologues (Figure 2). It is clear, however, that the ovine adenovirus genome shows major structural and sequence variations compared with all other adenoviruses studied to date (Figure 2), in the regions encoding both structural and non-structural proteins. In particular,

(a) the reading frames tentatively identified as forming the E1A/B regions are named principally on the basis of their location in the genome. Very limited homology can be detected between the 44.5kD open reading frame (orf) and the large T E1B protein of other

adenoviruses. Homology in the putative E1A region of OAV287 has not so far been detected;

(b) in other adenoviruses the E4 region is normally located at the right-hand end of the genome. The OAV287 E4? region is tentatively identified based only on the presence of a protein sequence motif HCHC..PGSLQC which is found in 18.8kD and 30.85kD orfs in this region. Identical or very similar motifs are found in the E4 34kD protein of human Ad2 and Ad40 and mouse adenoviruses;

(c) the distance between the end of pVIII and the beginning of fiber, which in other viruses defines the E3 region, is only 197 nucleotides (Figure 4). The E3 region equivalent, if it exists in ovine adenovirus, may consist of the cluster of open reading frames which are present in the right to left orientation on the complementary DNA strand, at the right-hand end of the genome (Figures 2 and 5). However, these sequences show no detectable homology with any other adenovirus and the functions of these proteins cannot be deduced from such comparisons;

(d) there is a region of approximately 1kb which lies between E3? and E4? which has a very high A/T content (70.2%) (Figure 1). As there are no open reading frames encoding greater than approximately 30 amino acids in length on either DNA strand it is unlikely that the region codes for any proteins, unless mRNAs are generated by very complex splicing events. This region has no known equivalent in any other adenovirus;

(e) other differences are apparent in the structural proteins of the virus. OAV287 lacks homologues of Ad2 proteins V and IX. However, OAV287 has a completely new gene coding for p28kD which is located on the complementary strand of the E1A? region (Figure 2 and 3). This is a structural protein with an apparent size of 28kD by SDS PAGE which, according to N-terminal sequencing data, is cleaved from a larger precursor. No homology

between this protein and others in the databases has been detected;

(f) in most other genomes the VA RNA genes are located between the Terminal protein and the 52/55k genes. In OAV287 there is no room for them as the reading frames overlap.

These differences serve to emphasize the unique character of the OAV287 isolate compared with other human and animal adenoviruses. In addition, since the OAV287 non-structural regions show little or no homology with equivalent regions in other adenoviruses, sequence comparisons do not reveal the identity of likely non-essential regions of the genome. Moreover the viral DNA cannot easily be manipulated to test for dispensable sequences.

The present inventors have produced a plasmid containing a full length infectious copy of an ovine adenovirus genome in which the ITR sequences are linked by a short sequence which creates a unique restriction enzyme site. A plasmid containing a full length infectious copy of an ovine adenovirus genome linked to a bacterial origin for DNA replication and a marker gene has been produced. Partial clones of OAV287 genomic DNA were specifically modified and initially linked to a gene encoding antibiotic resistance and origin of replication inserted into the unique SalI site of the genome (Figure 6 and see Methods). Such a plasmid can be grown in bacteria and more easily manipulated.

The circular genomic clone differs from the naturally occurring circles that occur in Ad5-infected cells (10) and that might exist in OAV287-infected cells in that the 40 base pair ITRs are joined by a GTAC linker. Together with the last and first nucleotides of the genome (G and C, respectively, see Figure 1), this sequence forms a unique KpnI site (GGTACC) when the ITRs are joined head to tail. Other sites such as EcoRI, BamHI, SalI, Kasi etc

which have recognition sequences beginning with G and ending with C are suitable if they are unique as the 3' and 5' terminal nucleotides of other adenovirus genomes are G and C, respectively. A plasmid with a suitable antibiotic resistance gene e.g. ampR and origin of replication can be inserted at the unique site or elsewhere in the genome to form a plasmid which can be propagated in bacteria. Plasmids propagated in the presence of 200 µg/ml ampicillin in *E.coli* strains JM109 and DH5-alpha retain the KpnI sites and inserted sequences, indicating that the OAV287 ITR sequences are stable when linked in this manner. This approach may therefore be used to engineer other adenovirus genomes. If desired the GTAC linker sequence can be removed and the authentic termini regenerated prior to transfection by digestion with KpnI (or another appropriate enzyme) and incubation with T4 DNA polymerase to create blunt ends (9).

A method for generating linear infectious genomes from circular plasmids involved digesting the circular plasmid containing the full length copy of the OAV287 genome with restriction enzyme KpnI to generate a genome with the authentic 5' nucleotide dCMP. The linear DNA is then introduced into CSL503 cells using lipofectamine as the transfecting reagent.

To develop a viral genome as a vector it is essential to identify region(s) of the genome which are non-essential for function. These regions can be then substituted or deleted to make room for foreign DNA (11, 12), or they may be the site for insertion of foreign DNA. In the human adenovirus genome DNA has been substituted or inserted into the E1 and E3 regions (13, 14, 15) and at the extreme right-hand end of the genome between E4 and ITR, usually with the concomitant deletion of non-essential regions to facilitate packaging of the genome (16). Adenoviruses will package genomes up to ~6% larger

than the wild-type, probably due to physical constraints dictated by the capsid structure (11).

Non-essential sites in the OAV287 genome were identified by insertion of a polylinker sequence  
5 containing *ApaI* and *NotI* restriction sites. This linker was introduced into the genome copy in pOAV100 between nucleotides 22,139 and 22,130 of Figure 1 by site directed mutagenesis to create plasmid pOAV200 (Figure 7). This corresponds to a site located in the intergenic region  
10 between genes for the pVIII and fiber proteins which was chosen because it avoids disruption of RNA processing signals in the region. A transcription termination site for the L4 family of RNAs maps 26 nucleotides upstream and the splice junction between  
15 the tripartite leader sequences and fiber mRNA maps 144 nucleotides downstream of the insertion site, respectively (17). Transfection of pOAV200 into CSL503 cells resulted in the rescue of virus OAV200. The second site at which the polylinker was inserted was located between bases  
20 26,645 and 26,646 of Figure 1. This created plasmid pOAV600 (Figure 7). This insertion site corresponds to the right hand end of the A/T-rich region (Figure 2) whose function and precise boundaries are unknown. The site was chosen as it is six nucleotides to the left of the  
25 transcription termination point for RNAs transcribed from right to left from the E3' region (Figure 2). This was determined by sequencing cloned RT-PCR-amplified cDNAs derived from the region using methods similar to those described for the pVIII/fiber region (17). Transfection  
30 of pOAV600 into CSL503 cells yielded virus OAV600.

The above insertion strategy identified two regions of the genome which can be interrupted and created sites for subcloning gene expression cassettes.

A further non-essential site was identified using  
35 the unique *SalI* site located at bases 28644-28649 of Figure 1. The site was cut with *SalI*, end-filled and

religated to disrupt the reading frames which spanned the site. A plasmid pOAV600S (Figure 7), which had lost the site was identified by digestion with SalI. When pOAV600S was transfected into CSL503 cells, virus OAV600S was recovered. The loss of the SalI site in this virus was confirmed by digesting the viral genome with SalI. As the SalI site falls within two significant open reading frames (which extend on the complementary strand between bases 28457 and 29014 and between 28511 and 28699), which were disrupted by end-filling and religation, the gene products derived from the reading frames are probably also dispensable. This group of reading frames may therefore constitute the E3 region of OAV287 as no other gene products in any adenovirus are dispensable for replication, in vitro. This implies that it should be possible to delete the whole region labelled as E3? in Figure 2. In addition, in other experiments a 1kb NdeI fragment was deleted from the region marked as E4? in Figure 2. This deletion disrupted several reading frames in the region. No virus has been rescued from a such a plasmid, suggesting that it is not dispensable and accordingly, it may be E4.

Many viruses replicate incompletely in heterologous hosts, often entering cells but being unable to produce mature virus particles because of a block in the replication cycle. In the context of recombinant viral vectors, this represents a desirable safety feature, provided that replication is not blocked before appropriate and effective expression of the foreign gene occurs. OAV287 does not replicate productively in heterologous cell types (18), the only exception so far being bovine nasal turbinate cells in which viral titres are significantly reduced compared with the CSL503 cells. Recombinant forms of OAV287 have been constructed to determine whether expression of a reporter gene under the control of an appropriate promoter occurs.

Foreign gene expression requires that the gene be functionally linked to a promoter. This may be a viral promoter inherent in the genome, or a foreign promoter subcloned together with the gene of interest into a suitable site. The promoter driving gene expression must function in CSL503 and preferably a range of other cell types. In this work an OAV287 genomic promoter was used initially. Subsequently an heterologous promoter was also used. In adenoviruses, expression of the structural proteins is driven by the major late promoter (MLP). Families of RNA transcripts derived from the MLP contain a common sequence element, the tripartite leader sequence (TLS) at their 5' ends. The present inventors have identified those nucleotides in the OAV287 genome which comprise the TLS by using RT-PCR amplification of late mRNA transcripts present in OAV287-infected cells and sequencing of cloned cDNAs (17). A candidate MLP was expected to be present just to the left of TLS exon 1 (Figure 2). The MLP and TLS elements were subcloned using PCR techniques into a separate plasmid pMT (Figure 9) and linked with genes of interest. These promoter/gene cassettes were subcloned as ApaI/NotI fragments into the polylinker ApaI/NotI sites of pOAV200. Using this strategy plasmids pOAV203, pOAV204, pOAV205 and pOAV210 were constructed. These incorporate genes encoding a 17kD soluble protein from *T. colubriformis*, a rotavirus VP7sc gene (19), the 45W antigen from *Taenia ovis* (20) and a membrane protein (PM95) from *Lucilia cuprina*, respectively. Plasmid pOAV202, contained the 17kD antigen but lacked the MLP/TLS elements. These plasmids were transfected into CSL503 cells and rescued as viruses OAV202, OAV203, OAV204, OAV205 and OAV210, respectively (Figure 10).

The human cytomegalovirus immediate early IE94 promoter plus enhancer, which functions in a range of human and animal cell types (21), was also linked to the

rotavirus VP7sc antigen gene. This cassette was assembled by replacing the MLP/TLS elements in pMT/VP7sc with the HCMV enhancer-promoter region. The cassette was inserted in pOAV200 to create pOAV206. pOAV206 was transfected  
5 into CSL503 cells and virus OAV206 was rescued (Figure 10).

CSL503 and other cells were infected with the viruses described above and at various times post-infection the cells were radiolabelled with  $^{35}\text{S}$ -  
10 methionine. Proteins of interest were recovered from cell lysates by immunoprecipitation using an appropriate antiserum. Recovered proteins were analysed by polyacrylamide gel electrophoresis and detected by autoradiography.

15 When virus OAV202 was used, no expression of the *T. coulbriformis* 17kD antigen was observed by immunofluorescence. As this virus lacks the MLP/TLS elements and carries only the 17kD gene this result demonstrates that there is no viral promoter upstream or  
20 adjacent to the insertion point between the pVIII and fiber genes which is capable of driving gene expression. As the E3 region is also missing from this site there is no requirement for a nearby promoter. This situation contrasts with observations made using a human Ad5 E3  
25 recombinant (21). In this case a promoterless gene inserted 3' proximal to the pVIII gene was expressed, probably from the adjacent E3 promoter or the upstream MLP (15, 21). This result further emphasizes the unique  
30 nature of the OAV287 genome. Recombinant OAV287 viruses carrying the MLP/TLS elements were tested for expression in CSL503 cells. With OAV204, expression was easily detected in infected, but not in uninfected cells at 24hr post-infection (Figure 11A). Similarly, when viruses  
OAV205, and OAV210 were tested, gene products of 24kD and  
35 approximately 95kD, respectively were detected (Figure 11B). Therefore it is clear that MLP/TLS elements contain



the necessary information to drive gene expression in the homologous cell line under replication-permissive conditions. However, when OAV204 was tested in a heterologous rabbit kidney cell line in which the virus  
5 does not replicate productively, no VP7sc expression was observed. Some replication occurs in bovine nasal turbinate cells, although to a lower titre than in CSL503 cells. In the latter cells, expression of VP7sc was detected following infection with OAV204 (Figure 11B).

10 Virus OAV206 containing the HCMV enhancer/promoter element linked to the VP7sc gene was used to examine the function of a heterologous promoter in the context of the OAV287 genome. CSL503 cells infected with this virus readily expressed VP7sc antigen at 24-48hr post infection  
15 (Figure 12A). With this virus VP7sc expression was also observed in the non-permissive rabbit kidney cell line and in bovine nasal turbinate cells (Figure 12B). These results suggest that the HCMV or a similar constitutive promoter may be preferred over the MLP to drive gene  
20 expression in OAV recombinants in non-permissive cells.

One recombinant virus was also administered to sheep. Five sheep were vaccinated intraconjunctivally and intranasally with  $0.7 \times 10^8$  pfu of OAV203. At three days  
25 post-inoculation virus was recovered from the nasal swab of one sheep and from the conjunctival swabs of two sheep and confirmed as the recombinant virus by PCR analysis. Animals showed no obvious ill effects from such  
vaccination.

The viral vectors of the present invention can be  
30 used for the delivery and expression of therapeutic genes in grazing animals. In species which are not normally infected by ovine adenoviruses the lack of pre-existing immunity should allow efficient infection, gene delivery and expression. The genes may encode vaccine antigens,  
35 molecules which promote growth in production animals, molecules which modify production traits by manipulating

hormone responses and other biologically active or therapeutic molecules. The virus does not replicate productively in many non-ovine cells but the use of heterologous promoters allows the delivery and expression of genes while minimising the possibility of virus spread to a non-target host. As the DNA of adenovirus vectors can persist in cells in an unintegrated form, with the appropriate choice of promoter, expression over a prolonged period can be achieved.

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20        It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments  
25 are, therefore, to be considered in all aspects as illustrative and non-restrictive.

## CLAIMS:

1. An isolated DNA molecule comprising a nucleotide sequence encoding the genome of ovine adenovirus (OAV287) substantially as shown in Figure 1 or a functionally  
5 equivalent nucleic acid sequence.
2. The DNA molecule as claimed in claim 1 such that the nucleic acid sequence encoding the genome of the ovine adenovirus is substantially as shown in Figure 1.
3. An isolated DNA molecule comprising a nucleic acid  
10 sequence encoding the genome of ovine adenovirus (OAV287) substantially as shown in Figure 1 wherein a portion of the adenoviral genome not essential for the maintenance or viability of the native adenovirus is deleted or altered.
4. An isolated DNA molecule comprising at least a 15  
15 nucleic acid base sequence being substantially unique to the ovine adenovirus (OAV287) nucleic acid sequence as shown in Figure 1.
5. The DNA molecule as claimed in claim 4 such that the  
20 at least 15 nucleic acid base sequence encodes a functional element of ovine adenovirus (OAV287).
6. The DNA molecule as claimed in claim 5 such that the functional element is selected from the group consisting of promoter, gene, inverted terminal repeat, viral packaging signal and RNA processing signal.
7. The DNA molecule as claimed in claim 6 such that the  
25 functional element is the inverted terminal repeat having the nucleic acid base sequence 1 to 46 as shown in Figure 1.
8. A plasmid including the DNA molecule as claimed in  
30 any one of claims 1 to 7.
9. A plasmid including the DNA molecule as claimed in any one of claims 1 to 3 such that the nucleic acid sequence encoding the adenovirus genome or a portion thereof is linked to a nucleic acid sequence encoding an  
35 origin of replication and a further nucleic acid sequence encoding a marker.

10. The plasmid as claimed in claim 9 such that nucleic acid sequences encoding inverted terminal repeats of the adenovirus are joined.

5 11. The plasmid as claimed in claim 9 or 10 such that the nucleic acid sequence encoding the marker encodes for resistance to an antimicrobial agent.

12. A viral vector comprising a DNA molecule including a nucleic acid sequence encoding the genome of ovine adenovirus (OAV287) substantially as shown in Figure 1 or  
10 a functionally equivalent nucleic acid sequence or a portion thereof and at least one nucleic acid sequence encoding a non-adenoviral polypeptide or polypeptides.

13. The viral vector as claimed in claim 12 such that the nucleic acid sequence encoding the genome of the  
15 adenovirus is substantially as shown in Figure 1.

14. A viral vector comprising a DNA molecule including a nucleic acid sequence encoding the genome of ovine adenovirus (OAV287) substantially as shown in Figure 1 wherein a portion of the adenoviral genome not essential  
20 for the maintenance or viability of the native adenovirus is deleted or altered, and at least one nucleic acid sequence encoding a non-adenoviral polypeptide or polypeptides.

15. The viral vector as claimed in any one of claims 12 to 14 such that the nucleic acid sequence encoding the polypeptide or polypeptides encodes a polypeptide or polypeptides derived from bacteria, viruses, parasites or eukaryotes.

16. The viral vector as claimed in claim 15 such that  
30 non-adenoviral polypeptide is rotavirus VP7sc antigen, the parasite polypeptide is *Trichostrongylus colubriformis* 17kD antigen, the *Taenia ovis* 45W antigen or the PM95 antigen from *Lucilia cuprina*.

17. A method of delivering a DNA molecule having a  
35 nucleic acid sequence encoding a non-adenoviral polypeptide or polypeptides to a target cell, the method

comprising infecting the target cell with a viral vector as claimed in any one of claims 12 to 16 such that the DNA molecule encoding the polypeptide or polypeptides is expressed and the polypeptide or polypeptides is produced by the target cell.

18. A method for delivering a DNA molecule having a nucleic acid sequence encoding a non-adenoviral polypeptide or polypeptides to an animal, the method comprising administering to the animal a viral vector as claimed in any one of claims 12 to 16, such that the viral vector infects at least one cell of the animal and the infected cell expresses the DNA molecule encoding the polypeptide or polypeptides and produces the polypeptide or polypeptides.

19. The method as claimed in claim 18 such that the animal is a grazing animal.

20. The method as claimed in claim 19 such that the grazing animal is a sheep.

21. A viral vector comprising a DNA molecule including a nucleic acid sequence encoding the genome of ovine adenovirus (OAV287) substantially as shown in Figure 1 or a functionally equivalent nucleic acid sequence or a portion thereof and at least one nucleic acid sequence encoding a functional RNA molecule.

22. The viral vector as claimed in claim 21 such that the functional RNA molecule is an antisense RNA molecule or ribozyme.

23. A method for delivering a DNA molecule having a nucleic acid sequence encoding a functional RNA molecule to an animal, the method comprising administering to the animal a viral vector as claimed in claim 21 or 22, such that the viral vector infects at least one cell of the animal and the infected cell expresses the DNA molecule encoding the functional RNA molecule and produces the RNA molecule.

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Fig 1

CTATTCATAT	ATATAACGTT	GCACAGAGGC	GGGGCGTGTG	GGTTTTTTAT	TGTTTTATTGT	60
CATGGAATTT	ACAAAGAAGT	AAGTTGTTGG	ATCTTTATTC	ACAATTCTTT	TAACAATGAC	120
TTTTTTACTT	ATTACATTTT	TCATCTTTTT	TACTTCACAT	GATATTTTAC	TTAAATTTTG	180
TACATACAAG	CCAAAATTCT	CATAAAATGT	CTTACTTTAA	AAAGTTAAAT	TTTTTTTTTTA	240
ACGCATAAAT	GGACGTACAG	CAGCAATTGG	AATAGCAGGA	AGGGCCATTG	TAAAGTGTGT	300
TCCTGCTGAT	CCCCTGTCAG	AAAGGATAGA	TGCTATCGTA	CGCATAAACC	CCCCTCCTAT	360
TTGTTTCATCT	GCTGCTTTTA	TTATATCTTC	TGCCAATCTA	GGTGATATTT	GCTTTTGAAT	420
GCTGTTTCCA	AAAGCTTGCA	TCATCGGATT	TTCAATTAAA	TGGATTGGAT	TTGCAGAATT	480
TCCTTAAAAA	TAGCCCAACC	CATCTAAAGC	AGTTAAAAGT	ATTCTCCCTC	CAGGAACCAC	540
AGATATAATT	AAGCGGAGCA	ACCGAGAGGT	TAAATTCCAG	GGTCCCTCGA	AGAGAGTATC	600
TAGGATCAGT	CCAAGAAGTG	AACCAAAAAG	ACTTGTAAGT	AGAAGTTGTC	TGATATGCTT	660
TGGAGAGGAC	TGTTAAAAAT	GCAAAAACGGT	ATCTAATGAC	CATTCTTCTC	TTACTTTTAC	720
ATCTGTATCA	TGTTCTCCAT	CAGAAGGTCT	TATTGGGAAG	TACCATTGGT	CACGAGCATC	780
TTTGAAGACT	TCTGTTTCTT	GAAATTCTGT	TTTCGGTAAG	CGACTAGCAG	TTATGGTATT	840
AGGAATATTG	ACGGTAATGT	TATTCACATC	TACAATTTCT	GGAGGAATCC	ATCTTGCATA	900
GGATGAAATG	GGTTTTGTGG	GTTCTTTCAA	TATATAATTG	CGAGGAGGGT	TTTTCCAAAA	960
TCTCTGAACA	TAAGTATTTT	CTGATTTTGG	CGGTTTTTTG	CTTTTTCGCG	CTCTTTTTCT	1020
TGGCTTTGGT	CTTTGAAATT	TTTTCTTCCT	TTTTCTGTAG	GCTCCTCCTG	CTAAAGCTGT	1080
GTTATTTGTG	ACGTACATCC	TGTTAGCTAC	ACGATTTTCC	CGGACTGCAA	ATTTTTTTGC	1140
CAAATGGAAA	AGAAATTGCT	GAAACCTTCT	ATTAATCATA	TAAATTGTCA	GTGGAATCAT	1200
GAATCAGATA	GTGCAGGATT	TTTTCTTTTT	GATACTGATA	ATTTATACTA	TTATGTATTG	1260
GATCAAGTGT	CTTGATATG	TTTAAGAGAT	ATAACTCTTC	ATTGTGATCG	CATGTGGTTA	1320
GCGGTTTGGT	TTTGTTTGTG	CAAATCTAAA	TTTGATGTAC	ACAATATTCT	AGCGGGAGTA	1380
CATGTTATGT	AATGAAAAATG	ACGTCGGGGA	TTGAATGGAT	TGAGCCTTAT	TTGACATTTT	1440
TCTGTGATTT	TTTTGCCCTTA	TTAGGAAATA	AATTTGTGGC	GCCAGTACGA	TGGAGATTGG	1500
AATGACTCCT	GCATTTACAG	AAAGGAATTT	GTACTGTGTT	TTGCTTGACT	TTAATTTAAG	1560
ATGGTATCAG	CAGATATTTA	ACCCAATATG	GATTAAGCCA	AATTTATGGG	CTTTCTCTGA	1620
TTTTTTAAAA	AAAATGGCCT	TTATTTATGC	TAGCGACTTG	GCGTTGTAA	ATTCTTACAT	1680
CCCTGGTAAT	GTTTGTAACA	AACTTGATAT	CATCAAGAAA	GATCTTCCTG	AAGATTTTAC	1740
CGTGTCTATG	TTTTGTGTCT	TAGTGTGTTG	GCTTGCTTCT	TTCTGTAAAG	GTTCTAATTT	1800
AGCTGAAACT	CGCCAGAATT	GTCACGCGGT	AAGCAAATTT	CTGGCACAAAC	TATCAAAATT	1860
AATAAAACCC	TAATTTTTAG	TTTGTAAGAA	TAGAATTCAA	ATTTTTAACG	CCACAATGAC	1920
TTCGGCGGAG	TTTTCTGTTG	AATTTCCCTA	TGTTTCTAAG	CCAATTGTTT	CATGGCCTGC	1980
TTCGGCATCT	TCTAATAATT	CATCGAGTCA	GAATATTGAC	TTTCCTGTTT	TTAAACCAGA	2040
TCAAGATCCA	ATAGCCTTCT	TTCAAACATA	CAATACGGCT	TACTTACAAC	CTGGAGCTAC	2100
TTATTACTGG	AAGTGATATC	AACTGTCAAA	GCCTATTAC	ATTTACGGTC	AAGGAGCTAC	2160
AGTACAACCT	GTCGGACCTG	GACCTGTGTT	TGTTTTCAAC	AGTGAAGTG	TTATTCCTGA	2220
AGATTTTTTAC	GTCGTGTTTG	AAAATATCAA	CTTTATTGAA	GATGAATTTT	CTATTAGAAG	2280
TGGCCAGTTA	AGTTTAGGAC	TTACAACCTA	CAGTGCTGTA	TGGTTTATCA	ATGTATGGAA	2340
AACTTCAATA	GTCAATTGTA	ACTTTAAAAA	TTTTAGGGGA	GCGGCTCTTT	GGTATTACAG	2400
TAATAGAAAT	TTTTGGAATG	CGAGAAAATG	GAATCAGCAG	CATTTAGTTT	CAAATTGTCTG	2460
TTTTAATGGT	TGTAGAATTG	GAATTTCTAA	TACTGGTTCA	TCTGAATATT	CCATAGCCAG	2520
TCAAAATCAA	TTTTATGATT	GTCAAATCTG	TTTTAATGTA	ACCGGGGGTA	ATTGGTCTAG	2580
AAATAATAAT	GTTATTGTTA	ACTGTAGATG	TGCTTATCTG	CATGTTGGAG	ATAACATGTG	2640
GTATGAAGGC	CATTCCGAAA	ATAATAATCC	CGCTAAGGGT	ACTTTCTGCA	ATAACATAAT	2700
TAACCATGCT	GATAACGGAG	GCAATGTCTG	GCTTACTCAG	TTTAAACTTA	CAGATGGATC	2760
AACGATACAG	TTAGCATCAT	TTTATTTTGA	TGATAATCAA	GAAATTCCAC	CTTGTTATAG	2820
CGGTAATTTT	CATTGGTTTG	GAGATGTAAA	CATTGTAAAT	TTTTCTACCA	CAAAAATTGA	2880
TAAATGGTGC	ATTACTGGAT	GTAATTTCTA	TGGTAATACA	CATGCAGCTA	ACGATGCTGG	2940
TCAAGTTTCA	GTTGCTGAAG	CTGTAAAAGA	CAAAGTGTTC	ATTATTGGGT	GTTCTGGTAA	3000
TAATGTAACC	ATGAAAAATA	TTGTAGAAGG	TAACATGACT	CCAAAAATTG	GTACAATAAA	3060
GTAAAAAACT	TTTTATTCAA	AACAAAATGG	ATTTACATTT	AAACGTTTTA	CATATTGATT	3120
CTGCGTATAA	GTTCTTTTTT	TAAACACTCT	TCTAATTTCC	ATACATGCTT	GATAAAACAA	3180

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Fig 1 (cont)

ACTTTGTAAA	TTCATAAATA	TAGGTTTGAC	TTGATCAGAA	GGTGAATAAT	AGCTCCATCT	3240
AAATGATTCC	GTAATAGGAA	CATTATTATA	TATTAACCAG	CTATATTTTG	AGTTAACTCT	3300
TGCATGATCC	ACTATATCTT	TAAGTACAGG	GATAAGTGCA	CTCGGAAATC	CAAAAGAATA	3360
GTTTTTAATA	AATCTATTTA	TCTGTGAAGA	ATCAAGCTGC	GGACTAATAA	CATGACATTT	3420
TGATTGAATT	TTTAAATCCT	TAATATTTCC	TCTATCATGA	CGCGGGTTCA	TATTATGTAA	3480
AACTACTACA	ACAGTGTAAC	CATTACATTT	GGCAAATCTA	TTAAAAATTT	TTGACGGTAA	3540
AGCATGAAAG	AAAGAACTTA	TAGAATGACA	TGATCCCAAT	TGATTTCATAC	ATTCATCTAT	3600
TATAATACAG	ATAGATCCTT	CACTTGCAGC	TCTGCAGAAT	ATATTATCTG	GATTATCAAT	3660
ATTTAGATTA	GTATCGGAAA	TAGCATCTTT	GAAAGCTAAT	TGTATAAATT	TTGGATTTAA	3720
TGTTTTTGTT	AGTGGATTAG	AGAATGCATC	GTAGTTTCCT	TCAACACACT	GTGCTTTCCA	3780
CGCAATTTTT	TCTTCTAATG	GAACAGTACC	TTTTTCTGGA	GTTATGAAAA	AAATTGTTTC	3840
TGGTATTGGA	TCAATTAGTT	TTCCAGATAT	AATATTTCTT	ATAAATTGAG	ATTTTCCGCT	3900
ACCTGTGGGT	CCATATACAG	TAACAATGAA	TGGTTGTAAT	CCGCAGTTTA	AACTGGGTAT	3960
ACAGCCATCT	TTTAACAGAT	TGTGAGCCTC	ATTTACAGTT	TTTTGATAAT	TTACAGCAAT	4020
ATTGTGTAAA	TCAGTCATAA	GTTGACCATG	ATACATACAT	TTATCAAAAA	CTTCTTGACT	4080
TTCTGGAAAT	GGATTTCTGC	AAATAGAAGG	ATCTATCTTT	ACAACATCAT	TTTTCCAATT	4140
TAATGTGTCA	CTTAAAAATT	TTCCCAAAAA	GGATTTTCTG	TCAATGGTTC	TTGCGGTCTT	4200
GGATTTGGGT	GTCTCTTGTC	GTACGGGTAA	AGTAAGTATC	CTTTCTTCCA	CTGGATCCCT	4260
TTCTCATCG	TTTGATCCTT	CCAAGGTCTC	AGAATTCTGG	TTAGTTGCTT	CTCTACCACC	4320
GTGAATGGTA	CATCGGTTCC	ACTTGC GGTT	TGCAGTGTCT	TTTTTAAACT	TTTCTCGAT	4380
GTCTGAACT	CTTTCTGTGG	TTGTTCTAAT	AAATTATAGT	CAGTAAACA	ATGTTT TAGA	4440
ATTTCATAGT	TTAAACAATT	TTTAGCATGA	CCTTTGGCTC	TTAATTTTCC	TTCTCCAATA	4500
AATTTACAGT	TTTTACAAGT	TATGCTTTTT	AAAGCATATA	ATTTAGGAGC	TAAAATACAT	4560
GTTTCTGAAC	TGAATGCTTC	AGCTCCGCAA	CGGTACAAA	CAGTTTCGCA	TTCAACCAAC	4620
CAAGTTAGAC	ATGGATGTTT	TTCATCAAAG	ATTAAATTTG	AGTTATATTT	TTTAAGTCTA	4680
TGTAATCCTT	TTGATAACAT	GAGTTGGTGG	CCCTTTTCTG	TTAAGAATAA	CGAGTCTGTA	4740
TCACCATAAA	TACTTTTTAT	CTCCCTTTCT	ATGTAAGGTT	TACCCATATC	TTCCCCATAT	4800
AAAATTTCTG	CCCACTCACT	CATGAAAGCT	CTGGTCCAAG	CCAGCACAAA	GGATGCTATC	4860
TGAGTTGGAT	ATCGGTTGTT	CTTGATCCAT	TCTTCCTTAT	CCTCAATAGT	TGTTAAAATT	4920
AAATCATTAC	AATCAGCAGA	TAAAAAAGTT	ATAGGCTTAA	AAGTCACGTG	ATCTTGATTT	4980
CCTATAAAAA	GTGGAAAATT	AAAATTTTCA	TTTGTGTCTT	TGGAATCTTT	GGGCGGCATT	5040
TCAGGTAGGT	TTGAAAAATA	CTGATTCCAC	TCAAATGAAC	GTTTTGGTAA	TGATTTACTA	5100
ATCACAGTTG	TGTATGATGT	AATTCAGCT	GATCCATTTT	CTAATCTTTT	TTTATCTTTC	5160
TCTTCAATAT	TTTCAGCAAA	CACTACTTTC	TTTTTATCTA	TACGGGTAGC	AAACGAACCA	5220
TATAAAGCAT	TTGATAACAA	TTTACTTATA	CTTCGCTGAA	TCTTGTTGTT	ACTTTTACTT	5280
GCTTTTTCTT	TAGCCATAAT	ATTTACTTTT	ACATATTTT	GACATAACGG	TTTCCAGTCA	5340
CTCCATACAG	CATACATTTT	AGAGCTTTTG	ATTATTTTGC	ATTTCCATCC	TCTATTGTGT	5400
AAGGTGATTA	AATCGATAGA	GGTCAGTACT	TCATTTATCA	ATGTTTCATT	TGACCAGCAT	5460
AACTTTCCAC	TTTTTTT TAGA	ACATAATGGA	GGTAACACAT	CAAGATAATC	TAATGATGGG	5520
GGTTCACAA	CGGCTACCAC	AATCATAGGT	TTGATTGAAT	TGTCAAAATA	ATCTATTTT	5580
TCTTTTCTTT	GTAGTAGTTC	TTGAAAGTAA	TCTATTTGTG	CATTGGCTTC	AAAAGCATT	5640
AAAGTTTTTC	CATATGGAAG	TGGATGCGTT	AAGGCACTAG	CATACATTCC	GCAGATATCA	5700
TACACATATA	TTGCTTCTTC	AAATATTCCCT	AAAAATGAAG	GATAACATCT	TCCTCCTCTT	5760
AAACTCATTC	TAACAAAATC	ATACATTTT	TCTGATGGAG	CTTCCAAATT	TCTTAGGAAT	5820
TCAGAGGGAT	GATCTTCTTC	ATTATAAAAG	ATTTGTTTAA	ACAATGCTTG	AGTATTACTA	5880
CTAATTGTAG	GACGTTGGAA	TATATTA AAA	GAACACTCAA	GCTTTAAAGA	TGTTGTACAG	5940
AACTCTTGAT	AACCTTCTAT	AAGTTTTTCA	ACTAATTGAG	CCGTAACAT	AACATCATCA	6000
ATACAATACT	CCTTAGCTTC	CTCTAATAAG	TTGTATTTT	GGTTGTGTTT	TGGTTGTTT	6060
TGTAAATATT	CTTCAAATGA	ATTCCAATAT	TTTTGAACTG	GATAACCATT	GTTTTCTTTT	6120
TCATATTCTC	CCAACATAAA	AAAATCATTG	ATTGCCCTGT	AAGGACAATA	ACCTTTGCTA	6180
ACACTCAACT	GATATGCAGT	AGCAGCGTCT	CTTAAAGAAG	AGTGGGTAA	CAAAAATGTA	6240
TCCCTAACCA	TAAATTTTAT	ACCTTGCCAT	TTCATATCTT	CAAAATTAAT	AATTCATTT	6300
TTCCATCTTT	CATAAGTTGT	ATGTGAAGGT	TTCTTAAAGC	AAGGATTTGG	AAGAGATAAT	6360



3/23

Fig 1 (cont)

GTAATATCAT	TAAATAACAG	TTTTCCAGCA	CGAGGCATAA	AGCTTCTTGT	CAGCTTAAAC	6420
ATTGAAAGTT	CTTCACTGTC	TATTCCTTCT	AATACATGAC	TTGCAAGTAT	GATTTTCATCA	6480
AAACCACAGA	TATTATGACC	TACTACATAT	AATTCAATAT	ATCTTGGTTC	GCACTGTTTT	6540
AATTTTTTTT	CTTTATTTAA	GACCATGATG	TCTTCATATG	ATAAATTTGA	TTCAAGACCA	6600
TGATTTTCAC	AAAACGTTGA	CCAGTATTTT	TTAGCTACTG	AAATTTGTAG	CTCTGTTCTG	6660
AATTTTTTAA	AAGCTATGCC	AATTTTCATCT	TCTTTTTTAT	TTAACATTAC	AAAACATTCT	6720
CTGTTTACCT	CATAACCTAT	ATCGGTTAGCT	ATTTTAGAAG	CAATTTTTTAT	GAGTGATTTA	6780
CATCCAATTA	ACTTAAAAAC	CAACAAGTAA	GGAGTAACT	GTTTTCCATA	CAAAGAATGG	6840
TAAGTATATG	TTTCAATATC	ATAAACAATA	AAAAGACGTT	TTGCTTTTAT	GGCTCCAAC	6900
GGATTAAATT	TGATTTTTTC	CCACCAGAGT	TTTGTTCAT	GGTGAATATT	GTGATAATAG	6960
AAGTCCCGTG	TTCTGGATGA	GCAGTTGTGT	ATATTACTAT	AAATTGTTCC	GCAGAATTCA	7020
CATTATTCTC	GTTGTTTAA	AGTTTTTATT	AAATATATTT	CTCCTTTTAA	AATCAATAAT	7080
TCTATTGGTA	ACAAATTTCC	ATTAAGAATT	TCTTCAGTCA	TCTTAAAAAA	TCTTTTGTG	7140
AACTTCCATA	TTTTTAAAGA	TACGGGGGTG	TTAGAATCAC	AAAGTTTTAA	AACATCTAAA	7200
ACATTTTCTA	CTTCTTGAA	AGAATTTAAT	TTTAAACCTT	GAATTGCAAA	GTAATTATAA	7260
AAACTTTTTT	CAAAATCTTT	GTAATATATA	ATTTTATAT	ATGTATCCTC	ATATATTCCA	7320
GTAATATAAG	TAGTAGTTCT	TTGCTTTATT	ATTGTCTTTG	AAGCCATCTG	TTTAAAGCCG	7380
CTTCCCGTAC	TCGCTCAAAG	CTTCTTAAAA	CAACTTCATT	TGTACTATAG	CCAACAATTC	7440
CAGACAATTT	TATTCTAAAT	GCTATTTCAA	CTGAATCTAA	ATCTGAAAAA	TCCGTGTTTA	7500
CTTGCTTGAT	TACTTCTTCT	ATGCTCCAC	TGCTTCTAC	GAAGTCTATA	TCTTGAAGTA	7560
ATTGGTCTCT	TTCTTCTGGA	GTTGAAAAAG	AGTAAGATCT	TTCATTAGCT	TCTATAATTC	7620
CTAAAAATC	ACGAGTTATT	CTGCTATATA	GTTGTCTGAA	TGCTTGTGTT	TCTCTATTAA	7680
ACCAAATCT	AGTAAATATA	TCTTCTCCAT	TTTCATTCT	ACCTCTTAAT	ATAATTTGAA	7740
CAAAATGGAT	TCCAATATTT	CTGGCAGCTA	ACCTATTTTG	CACTAAATTT	AAGTATAAGT	7800
AATATAGCGT	GCTTGCCACA	TGCTCTAATA	TAAAGAAATA	CACTAACCAT	TTTTGAATAA	7860
AATCATCAGT	CAATCTATTT	TCATTATAAA	ATCTAATAAG	TAATTGAAAA	AATTCACCTC	7920
CGTAATTAAT	AAAATTACTC	CTTCTTGCTT	CAGGAGTTAA	TTCTTCTTCT	AAATTTTGAA	7980
TTAAATCTAC	TATTGAAGCT	ATCACTTCAT	CATTAAATTC	TTCCCTACTC	AGATCGCTTG	8040
AGCTCGGCTC	GCGATCTGAA	AATCCTTCAT	CTTCTATTTT	AGGAACAGTA	AGAGGAGAAC	8100
TAGAAGTTTC	TTCAACATTC	CTTACCCTTT	GGCGTCTATT	AACAGGTAAT	CTATCAATAA	8160
ATCTTCTGAT	TACATCACCC	CTTGAACGTC	TCATTATTTT	AGTAATAGCT	CTATAATTTT	8220
CCCTAGGTCT	TAATCTGAAT	GGTAATCCTA	CTCTTGTCCT	TGACCTTAAA	GTTAATGCTC	8280
CACCATGCAT	CCCACCTTTT	CCTAAAGTTA	ATACAGTTGC	TAAATCTTTT	AAATTAATTC	8340
GATTTTCAGC	TTCTGGAATT	TCCAGCTGTG	AAAAATTCATC	TATAAAAAGC	TCAATCCAGA	8400
ATTCAGAAAA	AGGTAAGTCT	AATATACATT	CATAATTATG	CATGTTAGAC	AAAAATAAAA	8460
ATTTACATAA	AGCTTTTTTA	ATTTTACAAA	TTAACCTTTAT	AAGGTAAGTA	TCCCTTTCTT	8520
GCAAATTTAA	AACCATAAAA	GCTTGAGAAA	AAGGTTGATA	ATGCTGCTGA	AAAGATCTAT	8580
TCTGATTTTG	AGCTGAAATA	GCGGAGCCAA	AACCTTGCAAT	GTCTGCAAGT	TGCAGACTCC	8640
CTAATATTCT	ATCCATTAAT	ACCGCGTTTT	GAATTTGACT	AATTGTTTGT	GAAAAATTTT	8700
CTACATTTTG	AATTGCTCTC	ATATATGACC	CAGTATTTAT	GGAGTATGAA	CAATCAGTTA	8760
AAATTTGCCA	GGTCATGCGT	CTCTCAAAAC	TTATAGGTGA	AAGATACAAC	TTATATGAAA	8820
TGTTGCTGTA	AGTCCGCTGA	TCAAACAGAT	ACTGGTTTAA	AACCTCGCGC	ACATAAAAAAT	8880
ACCCAATTAA	TAAATTTGGT	GGAGGTTCTC	CTTCAAATGG	TGTTTGTGAA	GTAACAGGTC	8940
CTCTTGGGCG	TAAATCGAGT	AATTGAGTCA	CTGGATAATT	AAAAATCGA	TTAGCCCATT	9000
TTATTCCCTT	TTCATGTATA	GTCTTTGACC	TGGCAATACT	TCGATTATTA	AGGTCAAGTG	9060
TTAAACGTAA	ATATCGTAAG	GTATGTTGAC	TTTGCCAGT	GAGTTGTTGC	CATTGGTGAA	9120
TCTGCAAGGC	AAACAAAAAA	TTTATCTTAT	TACTGCAGAT	GCATCCTATT	TTACAAAAAT	9180
TACGTTTCAT	ATTGGAACCT	CCAGACTTAT	CAAGCAACTC	CCCGGGCACG	TCAAAATAAAA	9240
ATGAAAAAGA	TGAATTTGAA	CCAGCAGTTG	GCATTTCTAG	CAAACCATCT	GATGAATTTA	9300
ATATGAGACG	ATCTCAAAGA	GATGATAATT	TACCTAAAAG	TCAGATACCA	GATGATAGATA	9360
TACTACATGA	TAAAAATCCT	AAAATGGCAG	AAGAACGAGA	CTTAATGTAT	AAATCTTCTG	9420
CTTGCAATAA	ACTTGATGAT	TCTAAACAAT	TAAAAACTGA	TATGTTTCAGG	CCGGATTTTG	9480
CTGGAACCTAG	TCCAGCTCAA	AGACACATAG	AAGCCGCAGA	GCTAAAGAGA	AATGGATCTT	9540

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Fig 1 (cont)

ATACTCGTAG	TTTAGAACAA	TGGACACATG	ATTCTTTTAT	AAGTCATGTT	AAACAATTAC	9600
TTTCTAGACC	ATTTATATCT	CTAGGTATTA	CATATTTGGA	TGATTTTTTG	CAGACTTATT	9660
TAGATCATAC	TGAATCGTCT	TCTTTAAACT	TTCAACTGTT	TACTTTAATA	AATCACTGTT	9720
CAGAAAATAC	TTTAAAACGG	ATTTTAAAC	ACATTTCTAA	AAAAAATGAA	AAAAATCAAT	9780
ATGTAAATCA	ATGTTGATT	GATCTCATT	CATGTATATA	TCTAATTATA	AGAGATGAAC	9840
AAAATGTTAC	AGAACAAGTT	AATGCCCTTT	TAGTAAGTAG	TAATCACTTA	GCTTTACATT	9900
TTGCAAAGAA	AGCTACAGGT	GGATTCTATC	CTACAGCAGA	CAAGTTAGCG	AAGACTCATA	9960
TTTTTTTCAA	GAGAATAATT	TTAGGAATAC	TTTCGCTAGC	AGAAAGTATA	GGTTGCTATA	10020
CTGTGAATCC	ATATTGCAAA	AATCCTTTGA	AAAAGTCAAA	AGTAGAAGTA	GAACCAAGTG	10080
ACGAAATGTA	TATGTTTCAGC	TTAAAAGGTG	CACCTGAACA	TCCTGATTCC	GACGAAGACG	10140
AAGCAGTGG	ACTTCAAAAT	GAATAATTAT	CATAAATGGA	CTTCTAATGT	TATAGATGCA	10200
ATTCTATCAA	ACAAAGCTCT	TTTAGCTATA	AAAATTTTAA	AAGTCAACCG	TTTGCAAACA	10260
AATTGAATGC	TTTAGAATCA	GCAGTTGTGC	CTCCAAGAAA	AGATGATACT	CCTGAAATGA	10320
TAGCAAATCT	TTTAAAAGAA	TTAGTTGCTT	TGGGAGCTAT	TCGCAGTGAT	GAAGTTGGCC	10380
CATTATATTC	TGACCTTCTT	ATCAGAGTTC	ACAAATATAA	TAGCTTGAAT	GTTCAATCAA	10440
ATTTGCAAAAC	TTTAACAGGA	GACATTAAAT	CACCTCAATC	CGATATAATT	AGAAGTTCCG	10500
ATATTCCCAA	TTTAAGTAAT	CAAGTTGTTT	TAAATACATT	TTTAAATTCT	TTGCCCTCAA	10560
CTGTTACATT	TGGACAACAT	AATTATGAAG	CTTTTAAACA	AACTCTAAGA	TTATTTGTTA	10620
ATGAGACACC	TAATATTACA	GTTTTTAGAT	CAGGAAATGA	TACTTTAATT	CAGGTTAACA	10680
TAACAGGAAT	TCATACAATT	AATTTGAATG	ATGCATTTAA	AAATTTAAAA	AATTTTTGGG	10740
GAATAGTATT	AACAGGTGAA	TTTATTCCAG	GTGATATTAC	AAGCAGACTA	ACAGCTAATA	10800
CAAGAGTACT	GCTTTATTTT	CTTGCTCCTT	TTACAAATGA	TAATACATT	ACACCTGATA	10860
CTTTTCTAGC	TTTACTCATG	AAATTATATA	GATTGACAGT	TTCTTCTGCT	TTAGATTTTG	10920
AAGAAGAAAC	TGAAGCTGAA	GTAGAAAATG	TAGCTCAACA	AATAGGATCC	ACTAGTGCAG	10980
ATTTTACAAA	GACTTTAGGA	TATCTATTAA	AAAACAAAGA	AGAATCATTT	TCGCCTCCCA	11040
AATCATTATC	TCCTAGACAA	CTGGGTATTT	TAAGGTTTAT	ACAGAAAAGT	CTGGTAGATA	11100
AAATTGATAG	AAATAATGAA	GATCCATGGG	ATGCTTTAGA	AACTTTATCT	TATTCATTTT	11160
CTCCGTCATT	TTATGAGGCC	AATGGGCCTT	TTATTAGACG	GTTAATAACT	TATATGGAAT	11220
TTGCCTTACG	TAATTCTCCT	ACTTACTTCA	GAGAAATTTA	CTCCAACAAA	TATTGGATAC	11280
CACCCAATTC	ATTTTGGACT	CAAAATTATG	CAGACTTTTT	TTCGGAAAAG	AAAGAAAAAC	11340
AAAATTTTGA	AACATTTGAA	CCGCGGGAAC	TTCTTTTACA	AATCTCTGAG	GAAGAAGCTG	11400
TCCCGCATAC	AGAAGATTTT	CAGTCAGCCA	TCTCGCCCTC	TATGGGCCAA	ACTTCACTCC	11460
CTGCTCCTTC	TGTGTCAGAA	TACAGTAGCG	TGCCTCGGTC	AGCTTTTTAC	CCTCTCAGAG	11520
AACGTATCCA	AGAGAGCATT	TCAAAGGCAT	TCATCCCTCC	TTTGACAGGC	TATGTCGGAA	11580
AACAAATAGG	TGAAACTATT	TTCCCTGGTA	TGTGAGATCT	TGTAGCACCC	GCTGCGTCTT	11640
TAGTTGCAGC	ACAATTGGTT	GATTCAAGGT	TTAATAACAG	AAGACAAAGA	TTGAAAGACG	11700
CAGCCAGAAA	GCGTCACCGC	TATGTTAGAG	AGATGCATAA	TATTTCTGAT	AAAGAGTCAA	11760
ATGCTTCTAA	TGATACGGTA	ATATCACCTT	TGATTGGACA	TGGTTCGCGC	ACTGAAAATC	11820
GTTTTGAATA	TTTGAGACCT	AAAGGTGGAA	ATTATTTATA	CTAATAAAAA	TCATAACAGA	11880
CCTGACGGGC	GGTCATCCTT	TTTTATTAGA	TGCAGAAATT	TGTACCTCCA	CCACGAATCC	11940
TTGCTCCAAC	AGAGGGTAGA	AACAGTATTA	CTTATACGCC	TCTGGCACCA	CTGCAAGATA	12000
CAACAAAAGT	ATTCTTTATT	GACAATAAGT	CTTCGGACAT	TGAAAGTTTA	AACTTTACTA	12060
ATAATCACAG	TAACTTTTTT	ACAAATATTA	TTCAAAATGC	TGATTTGGCA	GCGGATGAAG	12120
CAGCAACGCA	AGATATTAAA	CTGGATGAAA	GATCTAGATG	GGGCGGTGAA	CTGAAAACCT	12180
TTATAAAAAAC	AAATTGCCCC	AATGTTTCAG	AATTTTTTAA	CAGTAATAGC	TTTCTAGCCA	12240
GATTAATGGT	AGATAAACT	GATCCAGAAC	ATCCTAAATA	CGAATGGGTA	CAAATTACAA	12300
TTCTGAAGG	CAATTGACCT	GGAAGCGAAC	ACTTAACAA	ACTTAACAA	GGTATTTTAA	12360
ACAATTACTT	AGAAGTGGGA	CGCCAAAAAG	GAGTAGAAAT	TGAAGACATA	GGAGTAAAT	12420
TTGATACAAG	AGATTTTTCA	CTTGATATG	ATCCTGAAAC	GGGACTAATT	ACTCCAGGAA	12480
AATATACATA	TAAAGCTTTT	CATCCAGATA	TTATCTTGCT	ACCTGAATGT	GGCGTAGATT	12540
TTACATATTC	TAGAATTAAT	AATATGTTAG	GTATAAGAAA	GAGATTTCCA	TATACTAAAG	12600
GATTTCAAAT	TTTATACAGT	GATTTGACGA	AGGGAAATAT	CTCTCCATTA	CTGAATTTAA	12660
ATAACTATCC	TCATTCTATC	GAACCTGTAA	TGCAAGACGA	AAATGGAGTT	AGCTATAATG	12720

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Fig 1 (cont)

TAGAAAAAAT	AAGTGACAAT	CCCCCAGAT	GGCAAACAAA	GTACAGATCT	TGGACTTTAA	12780
GTTATAAAAA	TAATGGAGGA	GCTAAAGCCC	TAAGTGTACT	AACTGTTCCG	GACATAACAG	12840
GAGGATTAGG	TCAAATTTAT	TGGTCAATGC	CAGATACTTT	TAAAGCACCT	ATTACTTTTA	12900
CTAACAATAC	TACAAAGCCA	GAAACACTTC	CAATTGTTGG	ATTACATATG	TTTCCTTTAA	12960
AAGCAGGGTT	AGTTCATAAT	ATAAATGCGG	TTTATTCTCA	ACTTTTGGAA	CAAATTACAA	13020
ATACAACCTCA	AGTATTCAAT	AGATTTCCTA	AAAATGCTAT	ACTAATGCAA	CCACCTTACA	13080
GCACCGTAAC	ATGGATAAGT	GAAAAATGTCC	CCTTTGTTGC	AGATCACGGG	ATTACAGCCAT	13140
TAAAAAACAG	CCTTACAGGT	GTACAAAGAG	TTACTATAAC	AGACGACAGA	AGGAGATCTT	13200
GTCCATACAT	ACAGAAATCT	TTGGCGACTG	TTGTCCCTAA	AGTACTTTCA	AGTGCTACAC	13260
TTCAGTAACA	ATCTGGCTGA	TATCTCTGGG	CCTTATCCTC	CTGGAACCGT	TATGTCTATT	13320
TTAGTTAGTC	CCTCTGATAA	TACCGGGTGG	GGTATTGGAA	CATCAAGTAT	GAGGGCTACT	13380
GGCTTGAAAT	TTTCTAAAAA	ACAACCTGTT	AGAGTGCAGC	CTTATTACAG	AGCTCAGTGG	13440
GGACAGCTTA	ATGCTCGTAC	TTCACTTGAG	AAACTAAAAA	CCAAATTGAA	ATATTATGAA	13500
AAATTGTACA	GGGACAGACT	AAAAAGAAAA	ACAGTTGTTC	CAAAGAAAAA	GAGGTCACCT	13560
ACATCTCCTG	CGGATCGACT	TAAAAAATAT	CTTAAAGCTG	TCAGTCAAAT	CAAAGCTTTC	13620
AATAGAGCTA	GAAGAGCAGC	CCAATAAATA	TTATTTTTCA	CTTGCAGATG	AAGGTAGTTC	13680
ACGTGCTTAA	ATCTCCTCAT	CGTCGAAGAC	ATACACGTCG	TTACAAAAAA	CTAAAAAAA	13740
TCAATCTATC	TCCATACATT	TTACCTAAAG	AATTGCAAGG	CGGTTTTTTA	CCAGCTCTCA	13800
TTCCTATCAT	AGCAGCCGCA	ATTAGCGCAG	CCCCTGCTAT	AGCTGGAAC	GTAATAGCTG	13860
CTAAAAATGC	TAATCGTTCT	TAAAAATTTAG	AAAACTTTTT	TTTTAACAGA	TCACATGGCT	13920
TTTTCAAGAT	TAGCTCCCCA	TTGCGGCTTA	ACACCTGTTT	ATGGCCACAC	CGTTGGAATC	13980
TGTGATATGA	GAGGAGGTTT	CAGCTGGTCT	AGTTTGGGAA	ATTCTTTTAC	TTCTGGTTTA	14040
AGAAACATAG	GTTCAATTTAT	ATCAAATACT	GCTCAAAAAA	TAGGTCAATC	ACAAGGATTT	14100
CAGCAAGCCA	AACAAGGTCT	ACTGCAATCA	AATGTTTTAG	AAAATGCAGG	ACAATTAGCA	14160
GGTCAAACCTT	TAAATACTTT	GGTAGATATT	GGAAGATTAA	AGGTAGAGAA	AGATCTAGAA	14220
AAATTGAAAC	AAAAAGTTAT	AGGGAACGAC	CAACAAATTA	CTCAAGAACA	ATTAGCTCAA	14280
CTAATAGCCA	GCTTAAAACC	AAAAGATGAA	ATGTTTGTA	AGCAATCAGA	AAAAATTGTT	14340
GAACCTATGA	GACCAGAAAT	TAAATCTAGC	CAAATGCCTG	TAGAAATGTC	TTTTTATGAT	14400
TCTGTAAGTG	ATGAACCAAT	CATAAAAACC	AAAGAAGTTA	GCCCTCCTTC	ATTTTCATCT	14460
GAATCTTCAC	ATTCATATTC	TCACCCAAGA	AAAAGAAAAAC	GCGTATCCGG	TTGGGGTGCA	14520
TTTTTGATA	ACATGACTGG	AGATGGAGTA	AATTTTAATA	CAAGAAGATA	TTGTTATTAA	14580
AAACACTTTT	TATTTACAGA	TGGAGCCACA	GCGTGAATTT	TTTCACATTG	CGGGTAGAAA	14640
TGCAAGGGAA	TACTTGTCTG	AAAATCTGGT	ACAATTCATC	TCTGCCACTC	AAAGTTTTTT	14700
TAATCTTGGA	AAAAAATTTA	GAGATCCTTT	TGTATGCTCA	TCGACGGGTG	TAACACTGTA	14760
CCGTTCTCAG	AAACTTCAAC	TTCGTATAGT	TCCGATTCAA	ACTGAGGACA	ATGAAAACTT	14820
TTACAAAAC	AGATTTACTT	TAAATGTAGG	AGATAACAGA	GTTGCAGATC	TTGGAAGTGC	14880
ATATTTTGAC	ATTGAAGGAG	TTATTGATAG	AGGACCTACT	TTTAAACCTT	ATGGAGGGAC	14940
AGCTTATAAT	CCATTAGCCC	CAAAATCAGC	TTTTCCCAAT	GCAGCTTTTA	TGGATACTGA	15000
TGAAGCTACA	ACAATTTATA	TTGCTCAACT	CCCTAATGCT	TATAATGCTC	AAAACAAAGG	15060
TGTAGAAGAA	GCAATTCGAG	TAGAAGCAAA	CACCTACTACT	CCTAATCCTC	AATCAGGAGA	15120
ATATGCTACT	TATGACTCTG	CCAAATTTAA	TCCAGAAACT	ACTGGTGCTT	CTGGAAGGCT	15180
TTTAGGAATT	AATAGCTTAG	GAGATCTTTT	TCCGGCTTAT	GGATCTTATT	GTAGACCTCA	15240
ATCAGCAGAT	GGTAACATTT	CAACTGCACC	CATAACTAAA	GTCTATCTAA	ACACTACTGC	15300
TACAGATGAC	AGGGTCAGTG	GAGTTACTGC	AGTTGACACC	GCAACCAGAT	TGCATCCAGA	15360
TGCTCATTAT	ATTGAATATA	CTGATGAAGC	CAAAGCTACA	GCTATAGGAA	ATCGCCCAAA	15420
TTATATTGGT	TTCCGAGACA	ATTTTATTGG	ACTCATGTTT	TACAATAATG	GTTCTAATGC	15480
AGGAACATTT	TCCAGCCAAA	CACAACAAC	TAATGTTGTT	TTAGACTTGA	ATGACAGAAA	15540
CAGTGAAC	AGCTATCAAT	ATCTAATAGC	AGATCTGACA	GATAGGTATA	GATATTTTGC	15600
ACTTTGGAAC	CAAGCAGTTG	ATAGTTACGA	CCAGTATGTC	AGAATTTTGC	ATAATGAAGG	15660
ATATGAAGAA	GCCCTCCGG	CCTTATCATT	TCCTTCTCAA	GGTATCCAAA	ATTATTTTCAT	15720
GCCTACTGCG	GCAGGTAATG	CGATGACAGT	AGACACGGGT	AGAAATACTG	CAGCAAAAAC	15780
AGATAACACC	AAGGCTTTTA	TAGGATATGG	CAACATGCCA	TCTTTGGAAA	TGAATCTGAC	15840
AGCAAATCTA	CAACGTACAT	TTTTGTGGTC	TAATGTAGCA	ATGTATCTGC	CAGATAGGCT	15900

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Fig 1 (cont)

GAAAAACAACA	CCACCCAACA	TAAATCTACC	TGATGACACC	AACTCTTACG	GATATATAAA	15960
TGGAAGGGTC	CCTCTAGCAA	ACATAATAGA	TACATGGACT	AACATTGGGG	CTAGGTGGTC	16020
ATTAGATGTT	ATGGATACTG	TAAATCCATT	TAATCACCAC	AGAAATTCAG	GACTAAAGTA	16080
TAGGTCACAA	CTGTTAGGAA	ATGGAAGATA	TTGCAGATTT	CACATTCAAG	TACCTCAAAA	16140
ATTTTTTCCT	ATAAAAAATC	TTTTGTTGCT	GCCAGGAACA	TATAATTATG	AATGGTACTT	16200
TAGAAAGGAT	CCCAACATGG	TTTTTCAGTC	TACTTTAGGT	AACGACCTTA	GAGCAGATGG	16260
CGCAACTATT	ACATACACCA	ACATAAATTT	ATATGTTTCA	TTTTTCCCTA	TGAATTATGA	16320
AACAGTAAGT	GAACCTGAAT	TGATGTTGCG	TAATGCTACT	AATGATCAAA	ACTTTGCAGA	16380
TTATTTGGGT	GCGGTAACCT	ATCTTTATCA	AATCCCAGCT	AATACAAATA	CTGTAGTAGT	16440
GAACGTACCA	GATAGATCTT	GGGGTGCTTT	CAGAGGATGG	AGTTTCAATA	GAATTAAAGC	16500
TTCAGAAACA	CCTATGATAG	GAGCAACAAA	AGATCCAAAT	TTTACTTATT	CAGGATCTAT	16560
ACCGCTACTA	GATGGTACTT	TCTATTTAAC	ACACACTTTT	CAACGAGTTT	CTATTCAGTG	16620
GGATTCTAGC	GTTCCATGGC	CAGGAGATGA	TAGGCTTTTG	ATTCCAAATT	GGTTTGAAAT	16680
TAAGAGAGAT	CCTAATATGG	ACGCAGAAGG	TTATACTATG	AGTCAAAGTA	CTATCACAAA	16740
AGATTTTTAT	TTGGTACAAA	TGGCTGCTAA	TTATAATCAA	GCTTATCAAG	GTTATAAATT	16800
GCCAGTACAT	TCTAAATATT	ATGGATTTTT	AGAAAATTTT	CAACCTATGA	GTCCGCAAGT	16860
ACCAATTTAT	GGTAATGGCA	CTTATGATTT	ATATACTGCT	TATATTACAA	ACCAAAGAAC	16920
CATGCAAAAT	TGGAATAATA	GTGGTTTAGA	ATCTAAAAC	TCAAATCCTC	CTATGTTATC	16980
CAACACTGGT	CATCTTTATG	TAGCTAACTG	GCCATACCTT	TTGATTGGAC	CAAATGCTAT	17040
TGAAAACCAA	CAAACTGAAA	GGAAATTTTT	GTGTGATAAG	TATATGTGGC	AGATACCATT	17100
TTCTAGTAAT	TTTTTGAAAT	TGGGTAATTT	AACAGATTTA	GGGCAAAGTG	TTTTGTACAC	17160
TAATTTCTAGT	CATTCACCTA	ATATGGTTTT	TACTGTGGAT	AGTATGCCTG	AAACAACCTA	17220
TCTAATGCTT	TTATTTGGTG	TTTTTCGACCA	AGTTGTTATT	AATCAACCAA	CAAGAAGTGG	17280
AATAAGTGTA	GCTTATTTGC	GCCTTCCTTT	TTCAGCTGGT	AGTGCAGCAA	CATGAGCGGC	17340
ACATCCGAAA	GTGAGCTGAA	AAATCTGATT	TCATCATTAC	ATTTAAATAA	TGGATTTTTG	17400
GGCATTTTTG	ATTGCAGATT	TCCAGTTTTT	CTGCAAAAAT	CTAAAATTCA	AACTGCTATT	17460
ATTAATACAG	GTCCCAGAGA	ACAAGGCGGA	ATACACTGGA	TAACATTAGC	ATTAGAACCC	17520
ATTTCTTATA	AGCTATTTAT	ATTTGATCCA	CTCGGATGGA	AAGACACTCA	ATTAATTAAA	17580
TTTTATAAAT	TTTCACTAAA	TTCTCTTATT	AAAAGGTCGG	CCTTAAATAA	CTCAGACAGA	17640
TGTATTACAG	TAGAAAGAAA	TACTCAAAGT	GTTCAATGTA	CCTGTGCGGG	ATCGTGCGGC	17700
TTGTTTTGTA	TATTTTTCTT	ATACTGTTTT	CACTTTTATA	AACAAAATGT	ATTTAAAAGT	17760
TGGCTTTTTT	AAAAATTAAA	CGGTTCAACC	CCTTCTCTGA	TCCCATGTGA	ACCACATCTA	17820
TTACATGAAA	ACCAGACATT	TCTTTATGAT	TTTTTAAATG	CAAAAAGTGT	TTATTTTCGA	17880
AAAAATTATA	GAACATTTAT	TGAAAATACT	AAGACTGGAT	TAATAAAAAAC	ACATTAATTG	17940
TATTCTTGCT	TTTTGACGTT	TTCATTAGTC	TTTCATCTTCA	TCTTCTTCTT	CACTGTAGA	18000
TTCCAAGATG	GTTTTTTTTT	TCTTTGATGG	AGTAGGCTCT	TCAATAGTTC	CAAAAGGATT	18060
CATATCAGAA	TCCTCTTCTA	TGTTAGGCAA	CATAGTATTT	TTAACCTGGA	ATGACTGATT	18120
CCACTTAAAT	TGAGAAAAC	GAATTGGAAT	GTTATTTCCC	ATACATTCAT	TCCAAAATTT	18180
ACGCACAAGA	GTTAAACACT	GTAACATATC	TGGCAAGCTA	ATTTTCATCT	CACAAAATTT	18240
TCCATTATTA	CGTCTCAAGT	TGTATTGATA	GTTACAACAT	TGAAACACAA	AAACAGCAGG	18300
GAATGTAAC	GCTGCGGCCT	GAACCTCTAT	AACATCCTGA	ACATCAATTC	CTTCCACTCC	18360
AGATATAGAA	AATGGAGTTA	TTTTAGGGAG	TTGTTTTTCT	ATTGTTTGTT	TGCCACCATA	18420
ATTACATTCA	CACTGACCCA	ATATAAAAAG	CATATTTCCG	ACTTTAGCTT	TCGGAAACAC	18480
AGCTTTTTGTA	GTTTCAATGG	CATTTTGCAT	AGCCAGCAAG	GCCTTCTTTT	CATCTGAAAA	18540
TTTAAGACCA	CAACTGCGAG	GAGAACATTG	CCCCAACGCG	TGATGGGCAT	CCTCAGCACA	18600
TAAACGTA	TGTTCTGAA	CTATTTTTAC	TACTTGTTTA	TTCATACGCC	CATTACTAAG	18660
AACACCCCTC	CCTTCCTTTA	GGGCTTGCAC	CCCTGCTTCC	GATGTTGGAG	GCATTTCAAT	18720
TTCATTCAAC	CTTTTAAACA	TGAAGTCACC	ATGAAAACAT	CTAGGACGGT	CCTCCTCCCA	18780
ATCATGATAC	CACAAATAAC	AACCAGAAGC	ATTAAAGTTT	GGAATCAAGT	CAATTTGCTT	18840
ACAAATTGCA	CTATATAGCA	TTCTACCTCC	TACAGTAGCC	ATAGATTTAC	TGCTACTATA	18900
AGTCAAATTT	ATAATTTTCA	TCTTTTTTCAT	GTACTGAGCA	AATAATTTT	CACAATCTCC	18960
TTCTTCAGGA	TGAAACTTCA	TTTGACTGGT	ATCAACTTTA	ACACACTCTC	CAAATTTAGC	19020
TAAAATTTTCG	AGCGCCGCTT	GAACCTTTATT	CTGAAATTCT	TCTGTAGTAG	ATTTTCTCTT	19080

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Fig 1 (cont)

CTTGATAGAT	TTAGTAACTT	TTTTAGAAGA	CATTATGTTA	GTTTTTTTCT	CGTTGTAGGA	19140
TGGCTGAAAA	AAATATGGGA	GAGTCAGAGA	AGGGTTTGAA	CGAAGAAGAA	TTTAACTCTA	19200
TTCTATCAAA	ACATCTGGAA	AGACAAATTA	AAATCTGTAA	AGCGTTAACA	TCAAAATTAT	19260
CGAACTGGAA	TATTGGAACA	TTGTTAGAAA	ACTTGTTATT	TTGTCCTGAT	GAAAGACAAT	19320
CATCAGGTGA	TCCCGACCCA	AAACTAAACT	TTTATCCGCC	TTTTTTAATT	CCGGAATGTC	19380
TTGCATTGCA	CTATCCATTT	TTTCTAACAA	CTCCTATTCC	GCTATCATGC	AAAGCGAACA	19440
AAATAGGAAC	TAACACTTAC	CGAAAATGGA	TGAACAATCA	AGTCCTGGAT	TTACAAATAC	19500
CTTCCTTGGA	AAATTGCAAA	TGGGATGATA	GCTTGGGAAA	TGTAGATTTA	ATTGAAGAGC	19560
TTAAAGAGAA	CCAAAAACTT	GTTTTAGTAA	AACAAGACCA	TGAAAGAAAT	ATATGGTTTA	19620
AATCAAAATG	CAAACTCACT	CAAAGTTTCA	GCTATCCCTC	ACTCAGTCTG	CCCCAGTTT	19680
TACAACAAGT	TTTAATTGAA	TCTCTTATCG	GCATTAGTCA	GGATCCTAAT	AACTTTGACA	19740
AAAATTACGA	ACCTGCAATA	ACTCTAGAAA	AACTACAACA	TGTAAACTGT	GATCAAGATT	19800
TAAAACAAGT	TCAACAAAAA	GTATCTTCAG	CCGCTACATA	CGGAATACTT	TTGAAATGCA	19860
TTCAGACTTT	ATTCAGTGAC	AAATTATTCA	TTCAAAACTG	CCAGGAATCA	TTACATTACA	19920
CCTTTAACCA	TGGTTATGTA	AAATTACTTC	AATTTTGTAC	AAATGTCAGT	TTAAGCGAAT	19980
TTGTAACCTT	CCATGGTTTA	ACACACAGGA	ACAGACTCAA	TAATCCGCAG	CAACATACAC	20040
AATTGGCAAC	CGAAGACAAA	ATAGACTATA	TCATAGATAC	AGTGTATTTA	TTTTTGGTAT	20100
TTACGTGGCA	GACAGCAATG	GATATTTGGA	ATCAAACATT	AGATGATAAA	ACAATAAATA	20160
TAATTAAGA	GGAATTA AAC	CAAAATTTTG	AGAAAAATGT	CAAAGCTGAA	TCAGTTGATG	20220
AAGTTTCTGA	AATTTTAAAG	TCTATTATTT	TCCCTGAACT	CATGCTGCCA	GCTTTTTGTT	20280
CTAATTTACC	TGATTTTATA	AATCAGAGTC	AGATATCAAA	TTTTAGAAAC	TTTATCTGCA	20340
TTAAATCCGG	CATACCGCAG	TCAATTTGCC	CCCTATTACC	TTCAGATCTA	ATTCCTTTAA	20400
CTTTCCTAGA	AAGTCATCCA	ATACTCTGGA	GTCATGTAAT	GTTACTAAAT	CTTGCTTCAT	20460
TTCTAGTAAA	CCAAGGCAAT	TATTTGCATG	AACCCGAAAA	ACCTTTAAAT	ATTTTCATCAG	20520
TTTACTGTAA	TTGTAATTTA	TGCTCTCCGC	AAAGAATGCC	ATGTTACAAT	AGCAGTTTGA	20580
TGCAAGAAAT	ACTAACCATT	GATAAATTCG	AGTTCACAAA	CTCTGATAAA	ACAAAACAGC	20640
TAAAACTGAC	CCTCCAAACT	TTTGCTAATG	CCTATCTTAA	CAAATTTAAC	TCAGCAGAAT	20700
TCTACCATGA	CCAAGTTTTA	TTCTACAAAA	ACTGTAAAAG	TAAATTTTCT	AACCAATTAA	20760
CAGCTTGTGT	AATAAAAAGAC	GAAAAATTAT	TGGCTAAAAT	AGCAGAAATT	CAAATAACGC	20820
GGGAAAAAGA	ACTCTTAAAA	AGAGGAAAAG	GAATTTATTT	GGATCCAGAA	ACAGGAGAAA	20880
TCTTAAACAA	TGGAGAAGCC	ATATCATCCT	CTGAAAACCT	CCAAAGGCAA	AGAACTAGCT	20940
ATGCTCTACC	ATCAAATGAA	GGAGAGCGAG	CTGGATGGGA	AGCCGATGAG	CGAAGAAGAC	21000
GAAGGAGAAG	TGAGTGAGGA	TGAAACAGAG	ACAACGAATC	CAAAGAAAAT	GAAGTTTACA	21060
AGTAAGTAA	CTCTAAATTT	TTTATATTAA	AAACTGAATT	TTTTTAGACA	AAATTATTTT	21120
AAATTAAATC	TTTATAGCTA	GCAGTTGATC	TTTGTTCGTT	TTTCAGAAAA	CTCAAGTGTT	21180
CAGTCATATC	AAGTTCACCT	GCCTCTGAAA	CACGAAATTG	CGGAAATTCT	AGAAAAAATT	21240
AGACTAGAAT	CTAAAAAATA	TCCAGGAAAA	GTTTATCAAA	TAAGAAATAG	AACTCCAGCA	21300
AGTATTACAA	AACGATACCT	GTATGAAAGA	GATCTGAAGA	AACTGTTCCA	GTATCTAGAA	21360
GACGCAAAGA	AGCTTTACGC	TAAGTACCAA	AGCTGAGGCT	TTATAGTTTT	AAATTTTCCC	21420
GCCATGGCTC	AACCAGTGAC	GCCTTACGTC	TGGAAATACC	AACCAGAAAC	AGGATATACT	21480
GCTGGAGCCC	ATCAAAATTA	TAACACTGTT	ATCAACTGGT	TGCATGCCAA	TCCACAAATG	21540
TTTGCCAGAA	TTCAACATAT	AAACACCGCA	CGCAATGTTA	TGGACAAATT	CCGCTCTGAT	21600
TTGACCCGAG	ATGACATCGC	GGTTAACATC	AACAACGGC	CTGCAGAGGA	TTTAATGCAA	21660
CCTCCTAATT	TTCTTACAT	TCCTGCGACC	TCTAAATCCG	CTTCAACCAT	AAATGACTGG	21720
TTGGCTACCA	CTCAAGGAAT	TCAACTCAGT	GGAACCTAGT	AACTAAACGG	GTGGGGATCT	21780
AACCGCCTGA	CTTCCTATCC	GGATATTCCA	CCCATTTTAA	AGTATGAAAG	GCCTGGTCAA	21840
CAACTTCAAG	GCCAAGGACT	TTTTAAGCAA	GAAAAATATC	ATTTATTTTA	CGAATCTCCG	21900
CGCCTCCCTC	GCTCTGGAGG	ATTAACCTCC	CAACAATTTG	TAAAAGAATT	TCCGCCTGTT	21960
GTTTATAATA	ACCCCTTCTC	AGAATCTATG	AGTGTATTTT	CGAAAGAATT	TAGTCCTTTG	22020
TTTAACCTTT	CAGAACTTTT	GAAAAAACA	TCCAGTCAAA	CTTTACAATA	TAAATAAAAA	22080
ACTTCTATTG	ATCTTTATAC	TTACACTAAA	GCATCGCGTT	TATTTTCGTC	GCCATAAAAA	22140
TATATCAAAG	ACCCGTAATT	CTCTAACTTT	AAATCATTTT	TTGAACATA	CTTAATCCAT	22200
TTAAATGTAG	GAATTAATAT	ATCAGAAACC	AGTAACAAGC	CAGAATTAAA	ATATACTTGT	22260

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Fig 1 (cont)

GTCATTTTTA	CAGATGAAGC	GAGCACGCTG	GGACCCGGTT	TATCCCTTTT	CTGAAGAGAG	22320
ACTGGTTCCT	CTGCCTCCTT	TTATTGAAGC	CGGAAAAGGG	CTAAAAAGCG	AAGGGTTGAT	22380
CTTATCTTTA	AACTTTACTG	ATCCTATCAC	TATAAATCAA	ACCGGTTTCT	TAACTGTAAA	22440
ATTGGGAGAT	GGAATATTCA	TAAACGGAGA	GGGTGGCCTA	TCAAGCACTG	CTCCAAAAGT	22500
CAAAGTTCCC	CTGACTGTCT	CAGATGAAAC	ATTGCAACTG	CTATTAAGTA	ATTCTCTAAC	22560
AACTGAGTCA	GACTCTTTAG	CTTTAAAAACA	ACCGCAACTT	CCCCTAAAAA	TAAATGATGA	22620
GGGGAGTTTA	GTATTGAACT	TAAATACTCC	TTTAAATCTA	CAAAATGAGA	GATTGAGTTT	22680
AAATGTTTCA	AATCCACTAA	AGATAGCGGC	AGATTCTTTA	ACTATAAACT	TAAAGGAACC	22740
CCTAGGATTG	CAAAAATGAAA	GTTTGGGCTT	AAATCTAAGT	GATCCTATGA	ATATAACTCC	22800
AGAAGGAAAT	TTAGGTATTA	AATTGAAAAA	TCCTATGAAA	GTTGAAGAAA	GTTCTTTAGC	22860
CTTAAACTAT	AAGAATCCTC	TCGCCATTAG	TAATGATGCG	TTAAGTATAA	ACATTGCGAA	22920
TCCATTAAC	GTTAATACAA	GCGGATCTCT	AGGAATATCT	TATTCTACTC	CCTTACGAAT	22980
TTCAAATAAT	GCTTTATCAT	TATTTATAGG	AAAACCTTTA	GGATTAGGAA	CTGACGGCTC	23040
TTTAACTGTA	AATTTAACTA	GGCCTCTGGT	ATGTCGTCAG	AACACTTTGG	CCATAAACTA	23100
CTCAGCCCCA	CTAGTGTCT	TGCAAGACAA	TCTTACTTTA	AGTTATGCTC	AACCATTAAC	23160
TGTAAGCGAT	AATTCTTTAA	GATTGTCTCT	AAATTCTCCA	CTAAACACAA	ATAGTGATGG	23220
AAAACCTTAGT	GTAAACTATT	CTAATCCTTT	AGTTGTGACT	GACTCTAATC	TTACCCTCAG	23280
TGTTAAAAAA	CCTGTAATGA	TTAACAACAC	AGGTAATGTT	GACTTAAGCT	TTACAGCTCC	23340
CATAAAATTA	AATGATGCAG	AACAGTTGAC	TTTAGAAACC	ACTGAGCCCT	TGGAAGTGGC	23400
CGATAACGCT	CTAAACTGA	AACTTGGA	AGGCTTAACT	GTTAGTAATA	ATGCTTTAAC	23460
CTTAAACCTT	GGAAACGGTT	TGACTTTCCA	ACAAGGTCTT	TTACAAATTA	AAACTAATAG	23520
CTCTCTAGGG	TTTAATGCTT	CTGGGGAATT	ATCAACAGCT	ACAAAGCAGG	GAACCATAAC	23580
CGTTAACTTT	CTAAGCACAA	CTCCTATAGC	TTTTGGGTGG	CAAATAATAC	CTACTACTGT	23640
AGCTTTCATT	TATATTTTAT	CAGGAACACA	ATTTACTCCT	CAATCCCCAG	TAACTTCTTT	23700
AGGTTTTCAA	CCCCACAAG	ACTTTTTGGA	TTTCTTCGTT	TTAAGTCCGT	TTGTTACATC	23760
TGTAACCTCA	ATTGTGGGAA	ATGATGTTAA	GGTTATTGGC	CTAACTATTT	CTAAAAACCA	23820
ATCTACCATA	ACTATGAAAT	TTACTTCTCC	CTTAGCTGAA	AATGTACCAG	TTAGTATGTT	23880
TACAGCACAT	CAATTCAGAC	AATGAATATT	TTAAAAATTC	TTTATTAAAG	AGTAATCTTT	23940
TTACATACCG	TTCTTGACAT	AATGTGCCCT	TATAATTAAC	AAATCTAAGC	AAGCAAGGTT	24000
GATCATTGGA	ATCTATAGAA	GCATAACTCT	TCCAATAAGC	ATAATCATAT	GGCGGTAAAT	24060
GAAAACCCCT	TAAATCTACC	ATATTCATCT	TTAAGTGTAC	AGTATCTAAC	AGGTTTTTAC	24120
AATCTTGAC	TTCTGGACTT	TTAAAAACAA	ACAGTACTTT	CATAGGACAA	CAATTGTAAC	24180
GGTTATAATC	TGTTACAATT	TTACTTATTT	CTTCTTCCAA	TGGCAAAGCA	TTCCAAAGTC	24240
TTGTTATAAG	TACTGTAAAA	TCATCAAAATG	AATAACATAA	CACATTTGTA	CAACAATTTG	24300
TCCAAGGTAA	AAAAACAGGC	ACACGAACAT	GAACTTTTTT	TAAAAATTAAC	ATCAGTGTCT	24360
GTTTTAAACT	TTGACATTGC	AAAGAATTTG	GCTGCAAGCA	ATGACAATGA	AATTGATTTT	24420
GCTGACAAGG	TAAGTCACAC	AAATACAAC	TTAACAGCCT	AAATATAACA	ACATTAATGT	24480
AACTTTCCAA	GACTTTAAAA	CTAACAAACG	GTATATCACA	ATAAAAAAGA	TGATGAATCC	24540
CTTCGCAACA	CATAATGGAG	TTCATGCTAC	ATCCAAAGAT	GGTTCCGACA	AACCTCTGTA	24600
AATTAAAGAA	CAACAATACA	ACATACGAAG	AAAATTAAAA	CGTTTTTTCAA	AACGAGATAT	24660
ACATTGCTGC	AAAGTATCTG	AACATTTACA	TTTTTACTTT	ATAAGCTCAC	AAGTTTCAGA	24720
AAATGTAATT	CGTTTAACAG	TTTGATATGA	ATACCATTTT	GAAGAAAAAT	AGAAATAGTT	24780
TTGTGCATTT	GTAAGCTCCC	AGAAACATTA	ACGGACAGGC	AAATCCAAGT	ATTACAACAA	24840
ACAGGAACAG	TCTTAACGTT	TCGTTTCAGAA	AACAAAGTAA	CAGGCATATG	ATTAAAGCAA	24900
GACAATAAAA	CACTTTTGGC	AGCTAAACAT	TGCAAAAGATC	CAGGTGAATT	ACAATGACAA	24960
TGATAATAAA	ACTTATAAGC	CATATCGGCC	CTCTTGCAAA	ACGAATCAGC	TTTTTGGCTT	25020
ATAGGAAAAA	AACAAAAAAA	CTGATTATAT	ATGAATGGAG	TTAATATCTT	CTTCAAAATTA	25080
TACACACGAA	TAGCAGAACC	AAGACGACCA	CGCCCAACAC	AGGTAAATAT	TTCAAGTCCA	25140
TGACTAGGAA	CAGATGGTTT	CTCACAAGCA	ACAACCTTTGA	TTTGCTTATC	CATCACTGCC	25200
AATCAGGCTT	AATAGGAAAA	GAAGAAAAAT	AATTTTCCCA	ATAATAACGA	AAGAAATTC	25260
ACGTTTCATC	CTGTACATTA	CTAGTCACAA	ATACAACCTC	CGCTATCAAA	GATTCCCTAT	25320
CATTTAAAAA	TCCCACCAAA	TTGTCCCAGT	CTACCTCAAA	AAAGCCAGTT	CCCATATTTT	25380
CAAAATTTGC	CCATTTTAAA	TAATCCAAAG	CATCAAATTC	AGGAAACAAA	TCTTTCTGAG	25440

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Fig 1 (cont)

CTAAACATA	TACAGTTT	TCGCCATT	ATCTAAAG	CATCCTAA	GGACCTCT	25500
CCCAGTAG	TAAGTACCG	GAAGAGACT	TACAATAT	TTGATATT	TGTCTGTT	25560
GTGGTGAT	AAAAGAAAG	AATTCAGAA	TAGGATAAA	CATTCTCCC	TGTTGATT	25620
TCTACAAAA	ACAAAAAAT	TATAAGGTT	ATAGAAAAC	TACTATTT	CAAACTCT	25680
AAAATGCAT	AAAAAGTT	CTTGAATAT	AATTCAGAT	ACCTAAAA	CGAAAAAA	25740
TAACATTT	GTTAGTAA	GATAGTCTT	AAAAATTAG	AAAGAATCA	GTCGCTTT	25800
TACTTACAA	CTCCAAATA	ATTCTGTA	CAAGAGAAA	ATTGTAAC	AAAAGGTA	25860
GAAGAACAT	ATAAGATT	AACCACTCT	AAATCTGAA	AGCATTAT	AAAAATCT	25920
TAGCTGCA	TTACTAGT	TCTCCAAAT	TTGCAGGCA	TTCAAAAA	CAAGAGGAA	25980
ACCGGAGTT	ATAAAGTAG	AGTCTGATT	TATCTGAAA	AGTTTAACT	CCTTTTCA	26040
CCAACCCAG	CCAATAAA	TCCAACCTT	ACTTCTTTC	TGCTAAAA	CCATAAAAG	26100
CCAAATTAC	CTTGACTTT	ATTTAACCT	AATTATGTT	CATGTTATT	TACCCATA	26160
AACTTGATG	CCAAGAACT	ACCTTTCCC	TGTTTTTCT	AAATAACAA	AATGTTGAT	26220
TAAAGATTT	TAATAACCA	AAAAACCCG	TCTCATGAT	TTTTCTTAT	TAAACAGGA	26280
ACAAAAGAA	TGGCAAAG	ATTCCATCA	ACTTCTCCA	CTGTCAAA	ATACCACT	26340
ACCTCTCCA	TGTTTTTTC	CTTTTGCAC	AACAGGAT	AAAAAATAT	TTTGCCACA	26400
TGTTTTTCT	TTTACTCA	TGCCAGAA	AAAATGAAC	GCTTAACCT	TTTCCCTCT	26460
AACCCATTG	GTTCTCTAA	GAAAAAAAT	ATCCCGCCA	ATATGCTAA	GGCTTCTCC	26520
GCCAAACAG	CTCAACTTA	AATCTCTCA	GAATAAAAC	CAGAGAAAA	TTCCAGTA	26580
AAAAATTA	AACCGTGA	TACTAGAT	AATAATGAT	TTTTGAACT	ATAAAAAAT	26640
ACCATCCAT	TAATGTTAC	AACACTTTT	TATTGAGTT	TTTCTTACA	CTGCATTAC	26700
TACAGGCCA	GCATCAAA	TTCTTCTGT	TTTCTTCTA	GACCACAAA	TTACAGACT	26760
ATATTTCTG	CACAAATCT	TATGATCTT	ACAGTAAC	TTACATTTA	ATGGGGAAT	26820
CAGCAGCAA	TAAGGATG	TTAAACATG	GATACAATG	CCAGAAGGA	GATAATACA	26880
TACATCAC	CAAAATGA	GTACAGACA	CATCGCATG	AATCTTAA	GTGATTTT	26940
AATAAATTT	TGCAGCAG	TACAATCT	ATTAGCAA	CGTTTTAT	ACAAACATA	27000
AAACTTGGA	CTTTTCAC	ACTCAATCA	GTTATTATA	CACATTACA	ATTTTGCT	27060
ATCTTTAT	GTCAAATA	AAAATATCT	AATCCACAG	TCATCTGG	GCAAACTTC	27120
CAAATCCAT	ACCTGTAAA	GATACAAC	AAAACAGAA	ATTAATGCC	TTCAATAAC	27180
TAAAAAAT	AGTCAAAT	CATACTTTT	CTCACTTAC	AAACTTTGT	AGCAGGCC	27240
CAAAACAA	TTCAGAAAT	GGATGCAT	AAGAACATT	TCCTCTCAA	AATTGCTTT	27300
ACTGAATGC	GCATTTTG	CCTCCAGAA	AATGCAGTC	ATTGAGAGG	TCTTCTCT	27360
AAACACAGA	ATGCTTCT	AAAATCTGT	AAGAACTAA	CAACTTCCA	ATTCCAAT	27420
TCATGCATT	CAAAGAAG	CATTCAAC	CAAAAGGAT	GTGATGAG	AATAAAGCT	27480
TACTGTATG	CTCATTTT	TGAATTAC	CTGTAACT	ACTATAAT	ATTTTAAG	27540
CTGCTTCAC	AATTAATA	GCTAATTT	TTAAGCAG	CAAAGAAAC	TCATCAGG	27600
AACGGCATT	AAGAAAGCA	CAAAATGAT	TCTTAAATA	CATTTTTC	GCATGATGA	27660
CAATAAAAA	TTTCAACGT	AAACAATGC	AAAATGCAT	TTTATGCAC	GTGAAAGTA	27720
TTTTTTTC	TGAAGCTAA	TCACAGCCT	TTTTATTAC	TGATTTTG	TGCTCCAAA	27780
GAGCTTGTT	TAATTGCTT	AAATCCAT	TCTTACAAT	TTTTCTTTT	ATAAACACC	27840
GAACCGCAT	CAGGCCAAT	CCAGTTATT	TTTAAATTT	CTACAGAA	TGCAGACC	27900
AAAACCAC	CCTCTAAAT	AACCCACAA	GATCTATG	CCACACAAA	ACACAAAG	27960
TGATACGG	AATACAACA	TAAATGGGG	TTAACAAGG	ACGCAACAC	ATGACCCGA	28020
GGTAATAAA	TTTACAGCA	CCAATTACA	GCAACAGGT	ATGGAGTAT	TTTCCCAAT	28080
CGACGAGAA	GCCGAATGT	ATTCAGAAC	GCATTGCAT	TTATCTTCT	AAACCTCT	28140
AGGTGCAAT	GTATAAAAT	AGAATCCTT	ATGACAGTG	TGAATTGAG	AAAAGCAAA	28200
ACAAAATAG	CAATTGCTT	GCTTGTAAG	TTCAAAAAT	TCTTCATCC	AATCTCAG	28260
GGTAATTCA	CAAAAAAT	AGGGGCCT	AAAATTAAT	AGACTAATT	AATATCAT	28320
TGTAAACAG	GAAAAGAAA	AATAACAC	CCAAAAATA	AAAACCTCT	CCCCTGTT	28380
CCATCGAG	ACACAGAAA	ATTCAGAAC	CTCAGTGT	TGTTTCTTA	ATTGTTCCA	28440
AAGCTCAG	ATTCTAAG	AAAAATTTT	TGAGAACT	AAAAACCC	TTTTTATA	28500
AAAGCCTTA	TGTTTTCT	ACTGATTTA	CTGCCCTA	AGGAACTCC	CATTCCGG	28560
ACGCCACCC	AGGGGACAA	TCTTGCCA	AACTACAAG	CCATAAAAC	ACATCCTG	28620



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Fig 1 (cont)

AATTATACCA	AAGGTTTCTA	TGGTCGACAC	AATTACAACC	TGACCTAAAA	GGTGAATAAA	28680
GCAGTAAATA	AGGATGAGTT	AAACAGGCCA	CACAATGTCC	AGAATGTAAA	AAATGCTTTG	28740
TTTGGCACCA	ACCAGACCAC	AGCTGAAGCA	AAGGAAAATT	GTAGCGAACA	CATTCTTCTC	28800
GTAATCTGTT	TAACACAGAA	CAACATTCAA	TTCTGGCAAA	CCTCTTTAAA	AAATGTTTTC	28860
TGAAATATTT	CTTTAAAATG	ACAGTTTGCA	ACTCTGGAAA	ACACAAAATA	AAAGCCGCAA	28920
TATCTCTACT	GCTTAAATAT	AAAAATATCA	TTGTCCAAAT	TTCTACTGGT	AAAAGTAAAA	28980
GCATCTTCTT	CCTATTAAAA	AAAGAAAAGT	GTTTTCAAAT	TATATTAGAC	TCTAACCAAA	29040
AAAATTCAAA	TACTTTTCCT	TTATAATGTA	CATTAGAAT	AAAAATATAC	TCACCGTTTA	29100
AAAGTAGAAC	TTAACAGTAT	AATATAAATA	CAAGTGAGCT	GAACAACGAC	AGCCGATTTT	29160
AGCCGGAGCA	AAATTAAAAA	GAATAAAAGG	ATCAAACCAA	CACGTAGGAC	AGTCTACTCC	29220
AAAACAGTAA	CGGCAGTATG	ACACAGAAGG	AGAGGAACTA	AGTCCAGGAA	ACTTCGCCCC	29280
GTGCGATAAA	AAGTAACGCC	GCCGGAAAAGC	AGTTGAATAC	AAAAGAGGTA	AAAATTCACG	29340
AAAAACAGAA	GCAAAAACTA	CTAAATCTGC	TATTGGCAAA	TAAAGAAAAA	TTTCAAACCA	29400
TATTTCCAAA	GGAAGAAAAG	CAATCATACC	GTAGAAGAAC	CTGAAGGCGA	CCGCAAACGT	29460
GCTCCCGTAC	CACAACGTCA	CACGCCACAC	CCACTGGGAA	AACCCACACG	CCCCGCCTCT	29520
GTGCAACGTT	ATATATATGA	ATAG				29544



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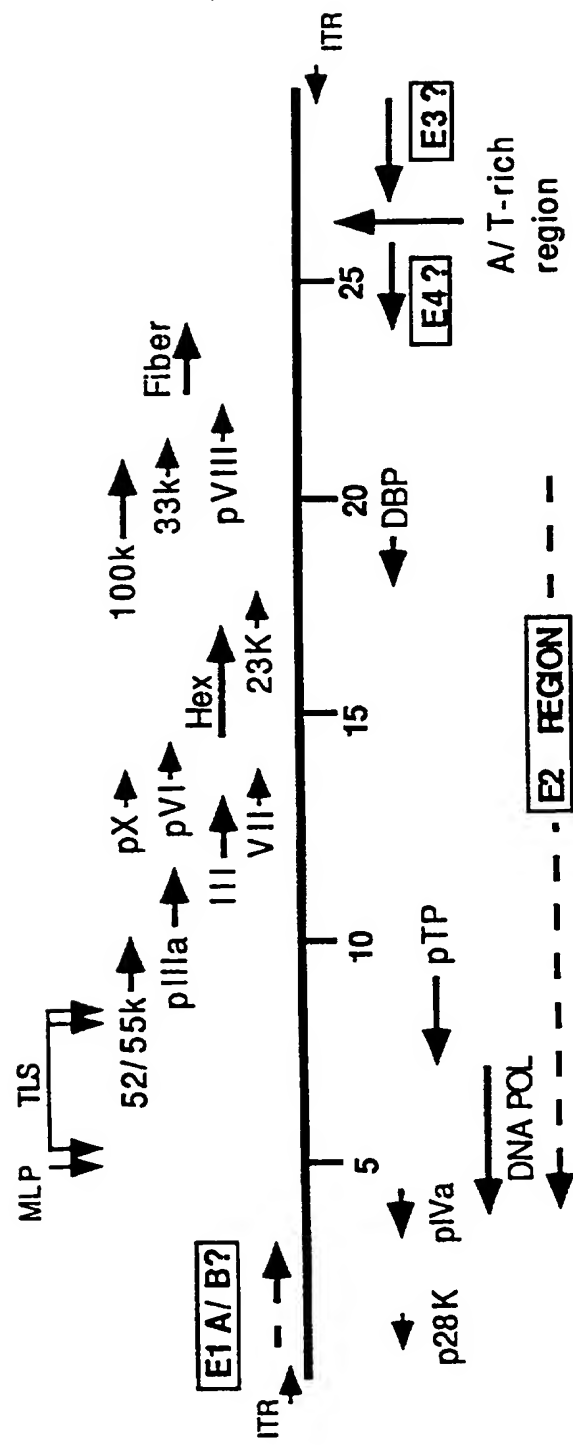


Fig. 2

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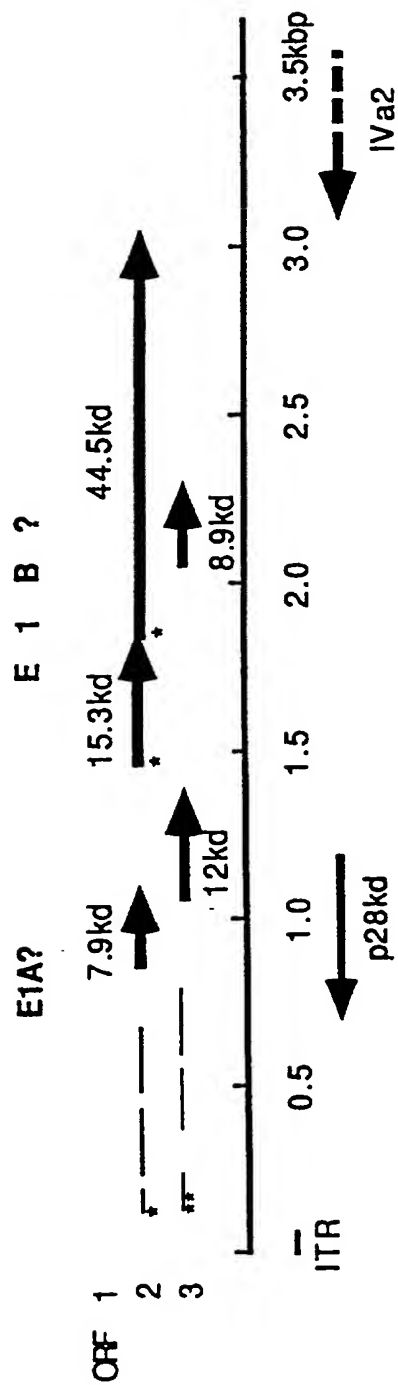


Fig. 3

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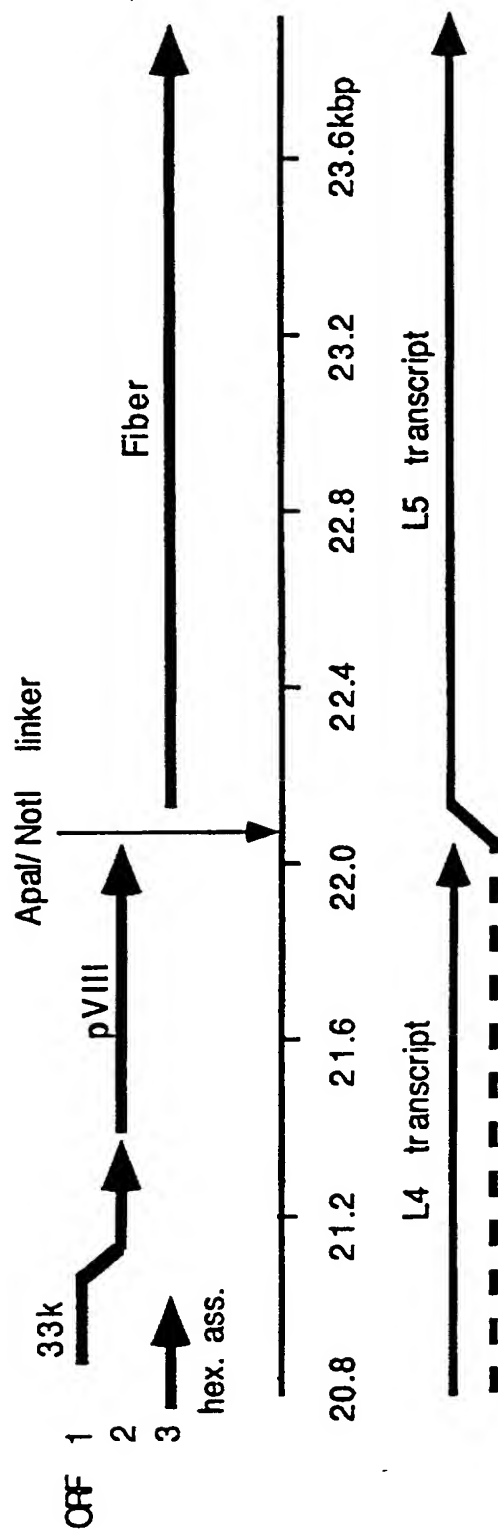


Fig. 4

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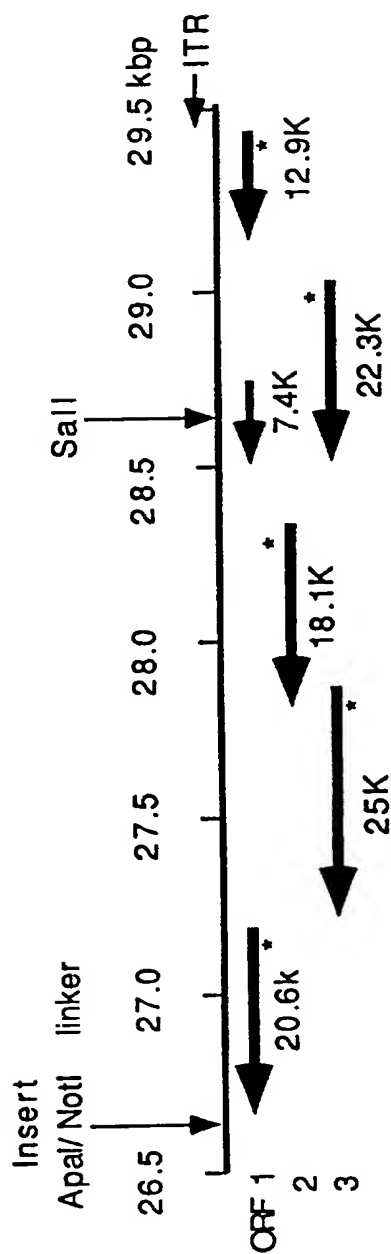


Fig. 5

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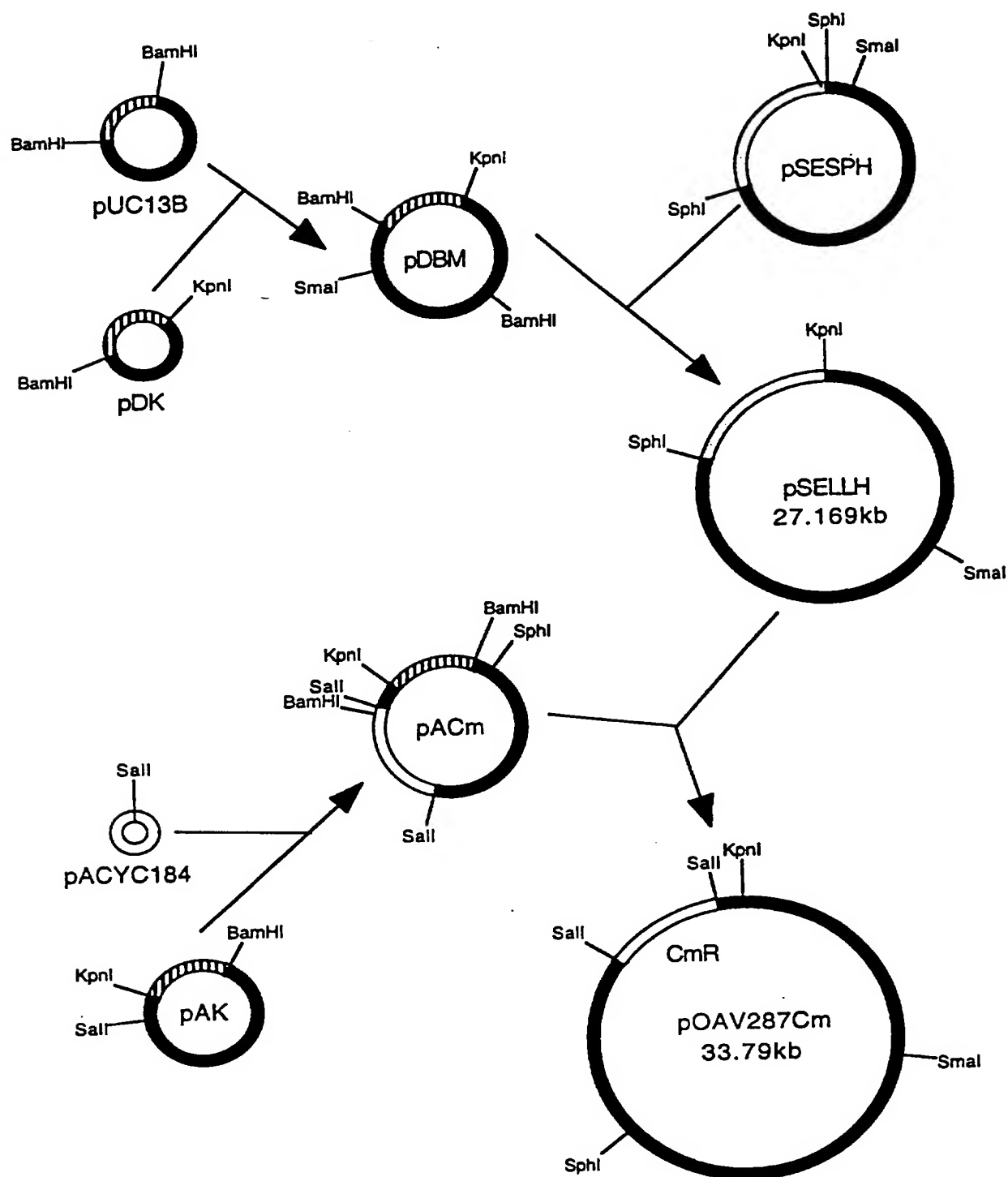


Fig. 6

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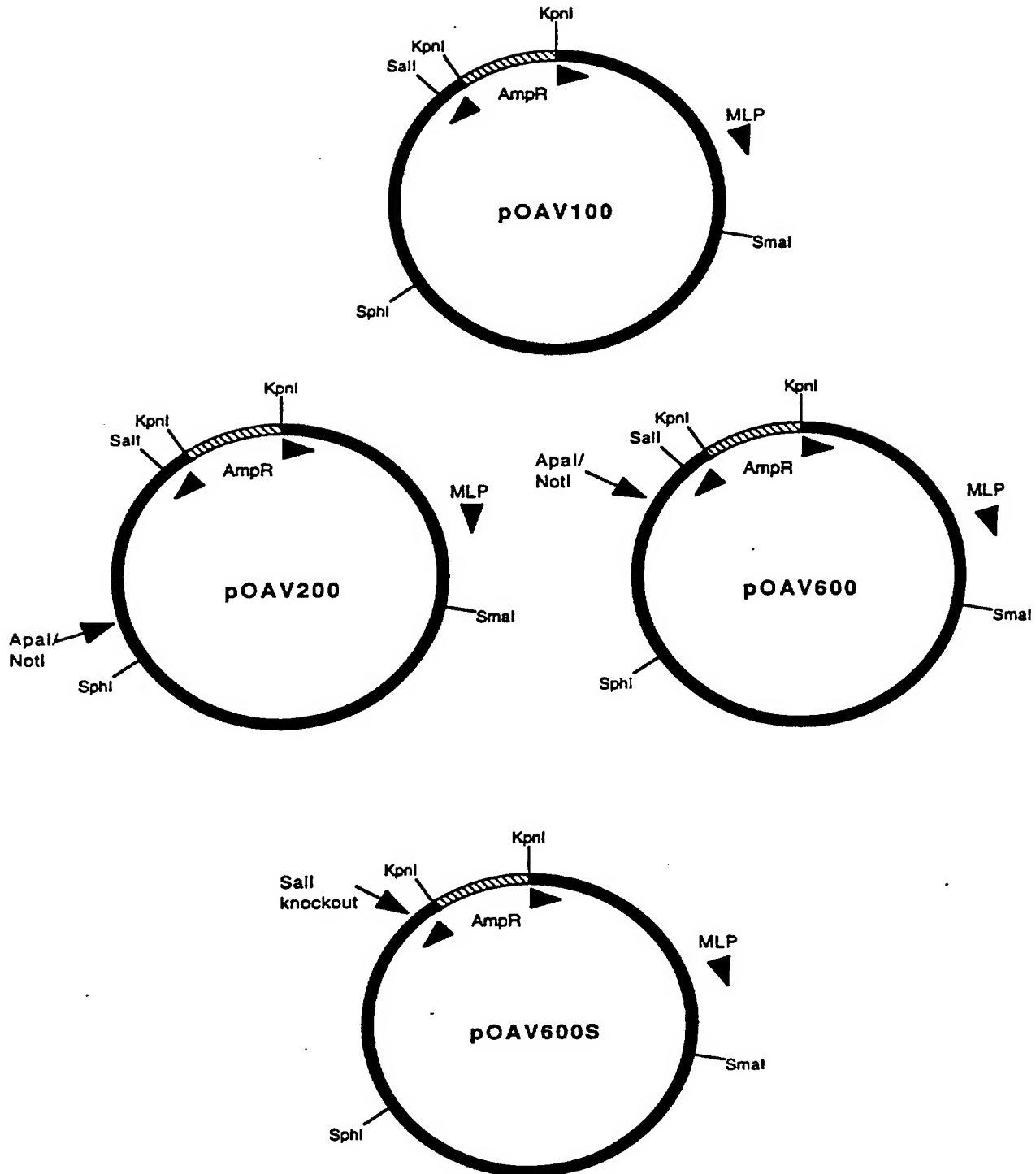


Fig. 7

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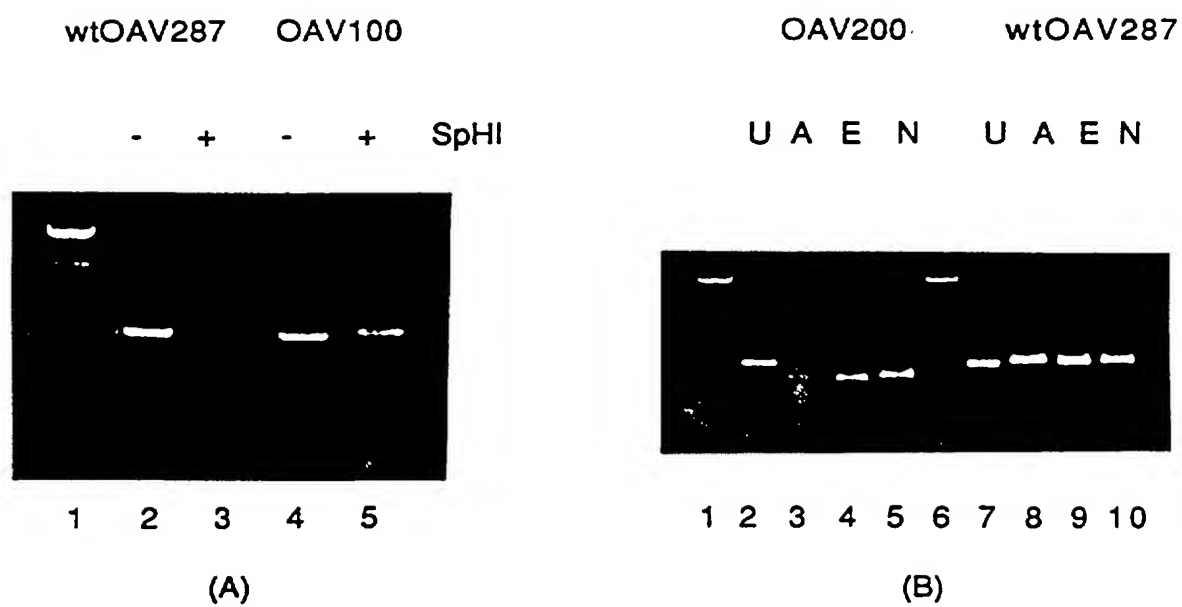


Fig. 8

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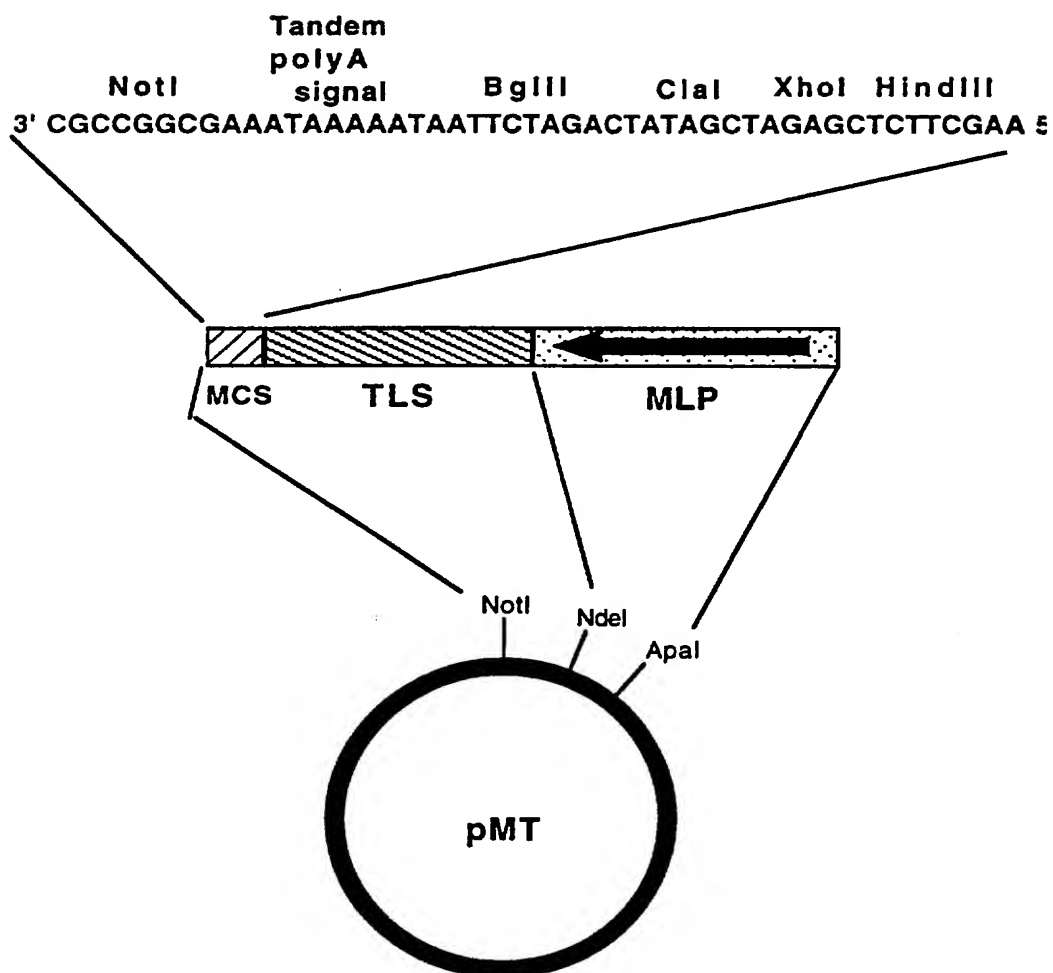


Fig. 9



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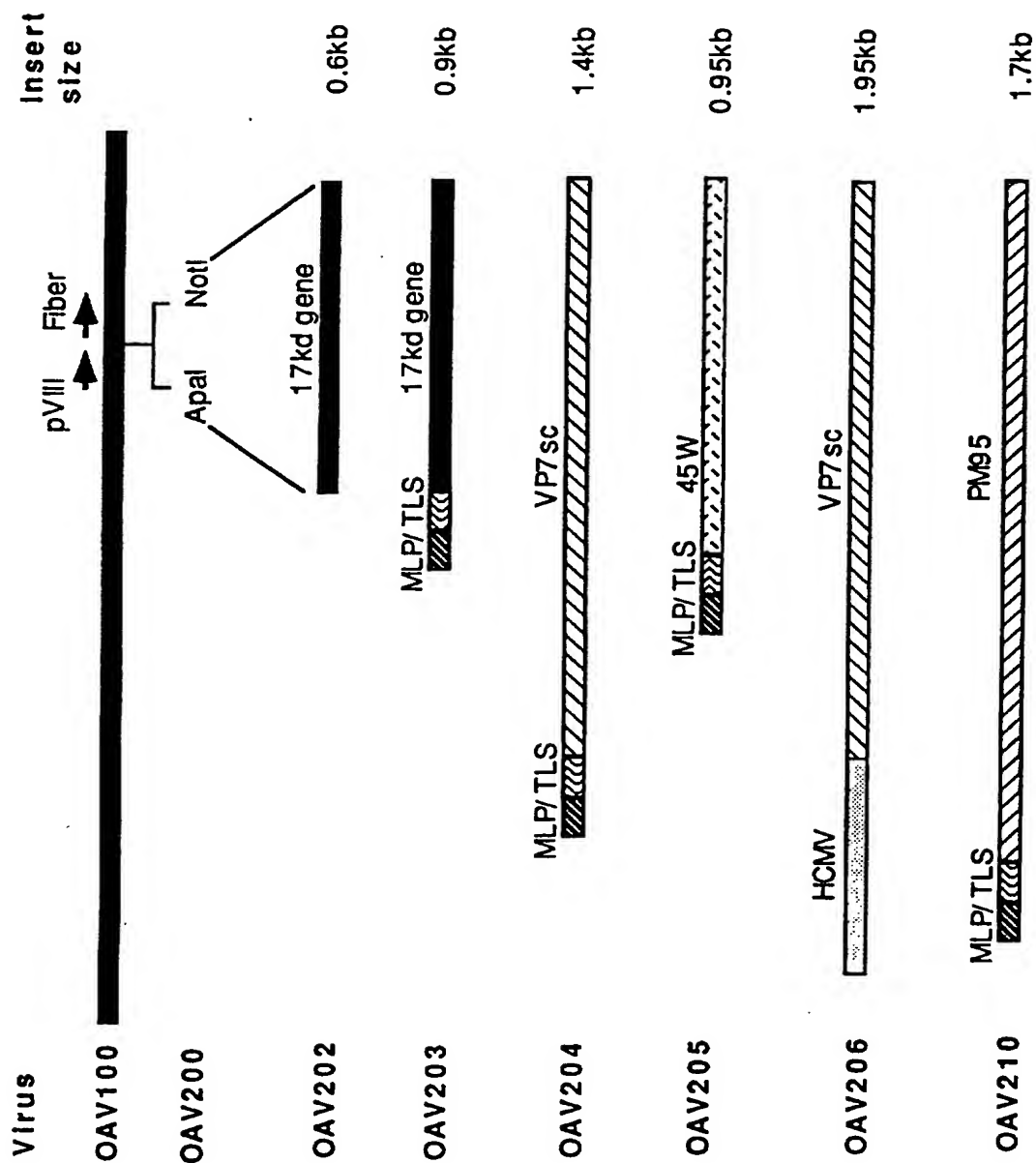


Fig. 10

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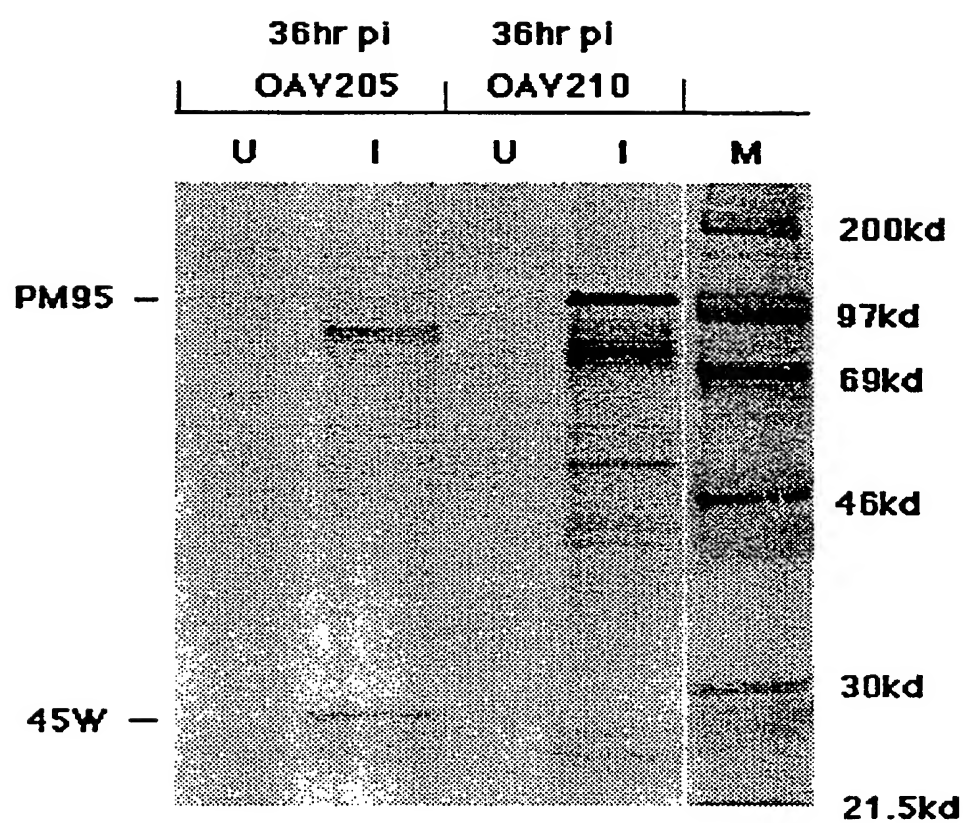
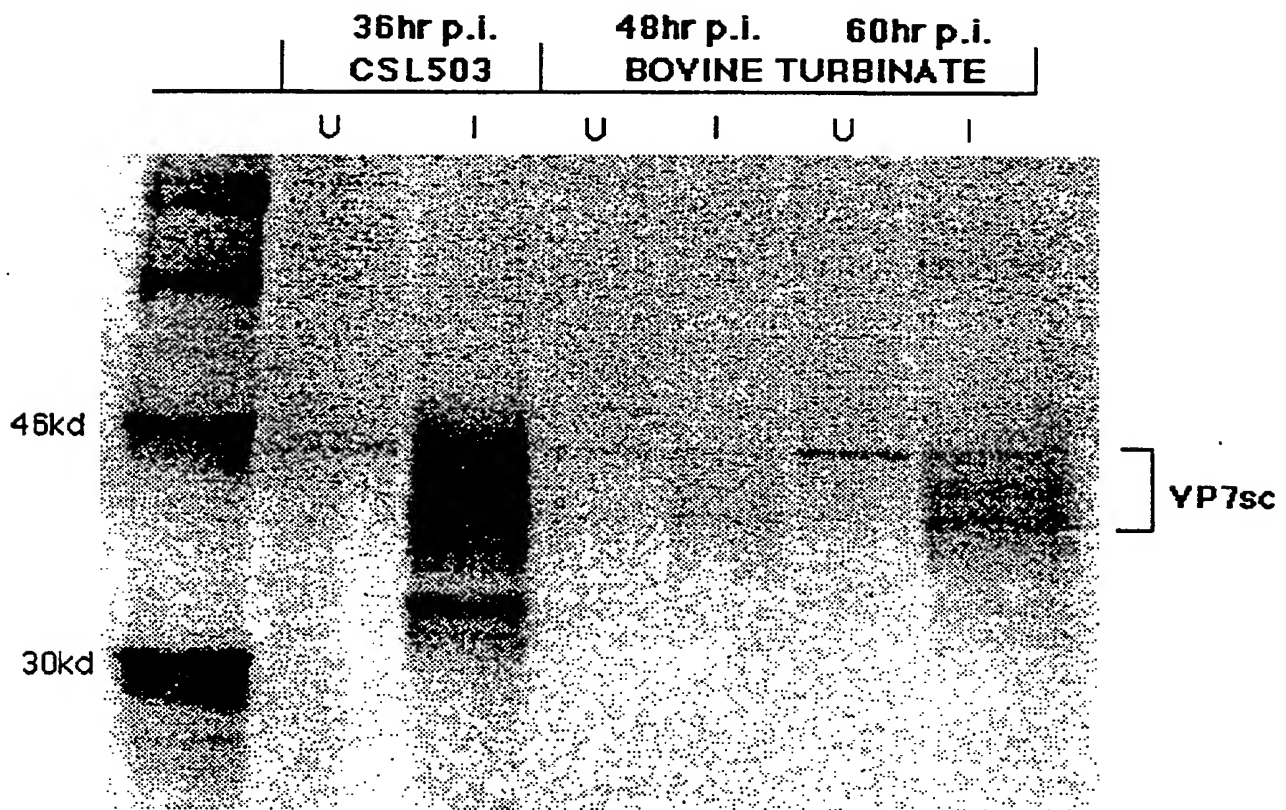


FIGURE 11A

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Infection with OAY204 (MLP/VP7sc)

FIGURE 11B

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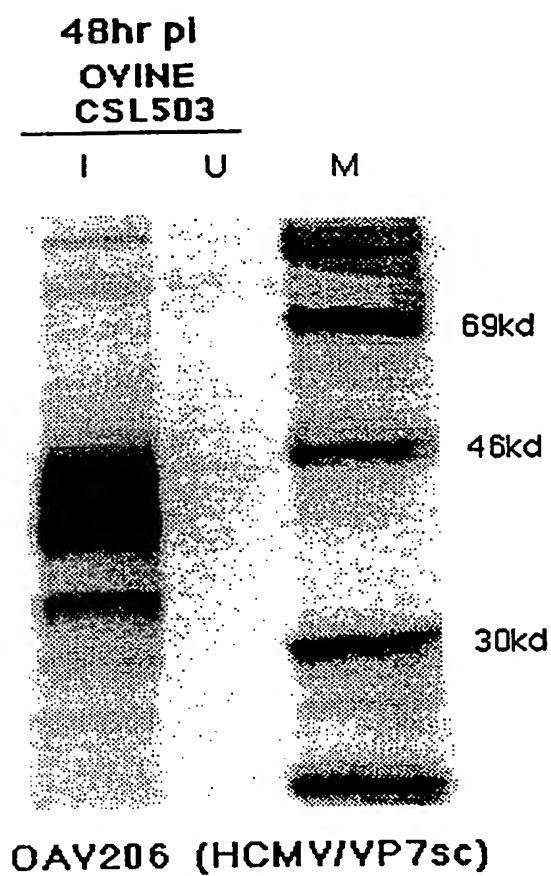


FIGURE 12A

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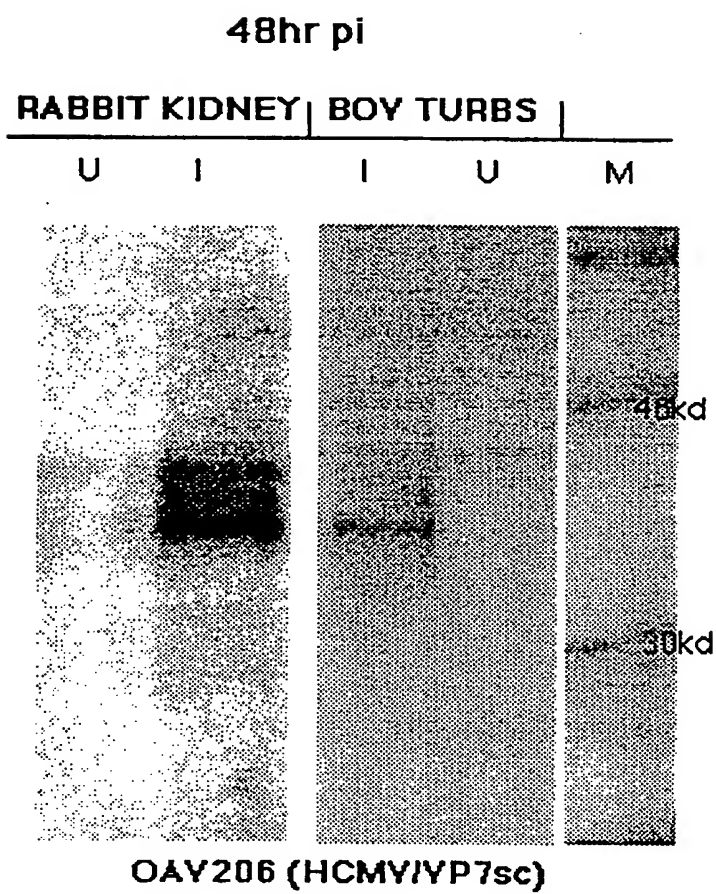


FIGURE 12B

# INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/AU 95/00453

## A. CLASSIFICATION OF SUBJECT MATTER

Int Cl<sup>6</sup>: C12N 15/34, 7/01, 15/86; A61K 48/00, 39/235

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC<sup>6</sup>: C12N 15/34, 7/01, 15/86

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DERWENT: ovine, sheep, ewe, adenovirus, adeno (w) virus, vector, trichostrongylus or colutoriformis or taenia or ovis or lucilla or cuprina

CHEMICAL ABSTRACTS: STN sequence search

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
<u>P,X</u>	VETERINARY MICROBIOLOGY, Volume 41, No. 3 (1994), BOYLE, D B et al. "Characterization of Australian ovine adenovirus isolates", pages 281-291 whole document	1-23
A	NICHOLSON, B H "Synthetic Vaccines" published 1994 by Blackwell Scientific Publications (Oxford, UK) pages 346-361 whole document	1-23
A	JOURNAL OF APPLIED PHYSIOLOGY, Volume 76, No. 6 (1994) LEMARCHAND, P, et al. "In vivo adenovirus-mediated gene transfer to lungs via pulmonary artery", pages 2840-2845 whole document	1-23



Further documents are listed in the continuation of Box C



See patent family annex

### \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance  
 "E" earlier document but published on or after the international filing date  
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 "O" document referring to an oral disclosure, use, exhibition or other means  
 "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  
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 "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art  
 "&" document member of the same patent family

Date of the actual completion of the international search  
26 October 1995

Date of mailing of the international search report  
6 NOVEMBER 1995

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